JOURNAL OF DAIRY SCIENCE

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Vol. XXVII, No. 3, March, 1944

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JOURNAL OF DAIRY SCIENCE

VOLUME XXVII MARCH, 1944 NUMBER 3

RELATIONSHIP OF FAT ACIDITY TO RANCIDITY IN HOMOGENIZED RAW MILK*

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The action of milk lipase on butter fat is generally believed to produce at least two detectable changes: first, the production of free fatty acids, and second, the production of rancid or rancid-like flavors. The production of the rancid flavor is attributed to free butyric acid and other lower fatty acids such as capric, caprylic, and caproic. Furthermore, the appearance of the rancid flavor is expected whenever milk fat undergoes lipolysis, indicating that the glycerides of the lower fatty acids are always attacked by lipase under normal conditions. Some efforts have been made to correlate the extent of fat splitting with rancid flavor development (1, 5, 7), but information on this relationship is by no means complete nor has it been applied to conditions in which the lipase activity is accelerated by homogenization.

The amount of lower fatty acids liberated from butterfat by lipase action may be expected to be relatively small inasmuch as the glycerides of the lower fatty acids constitute a comparatively small portion of the total fat and, also, since the work of Willstatter and Memmen (10) indicates pancreatic lipase to have slight affinity for the lower esters.

Although the quantity of the lower fatty acids involved in lipase activity is small, the effect of these acids on flavor may be marked. Grossfeld and Battay (3) observed butyric acid could be detected by smell when present in a ratio of 1: 12,500. In the case of dairy products, the actual type of flavor produced by lipase activity may not always be well defined and may vary with conditions. Relatively slight lipase activity may result in flavor defects which are not typically rancid (4, 7).

Results which have been reported showing the relationship between the acid degree of the fat (ml. of N NaOH per 100 grams of fat) and the rancid flavor in the original product from which the fat was obtained are not in total agreement. Fouts (1) in his study of commercial butter, could find no direct relationship between rancidity of the butter and the acid degree of the butterfat. The acid degrees of fat from non-rancid butter ranged

Received for publication July 24, 1943.

* Journal Article No. 656, new series, Michigan Agricultural Experiment Station.

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from 2.9 to 11.6, whereas values for rancid butter ranged from 2.4 to 14.0. In those samples which were rancid, there was no relationship between intensity of rancidity and free fatty acid content. Recently, Krukovsky and Herrington (6) and Jack, Tarassuk, and Scaramella (5) conducted controlled experiments in which butter was manufactured from milk having naturally active lipase. Krukovsky and Herrington found the rancid flavor to appear in butter between acid degrees of 0.75 and 2.0 with the lower value being near the flavor threshold of rancidity. Jack *et al.* concluded rancidity of butter was recognized organoleptically when the acid degree approached a value of 2.0 and that direct correlation exists between acid degree and rancid flavor.

In experiments with milk, Krukovsky and Herrington (7) determined "the acid degree at which rancidity could first be recognized as such." Although some variations occurred, the threshold at which rancidity was recognized in the milk was approximately at an acid degree of 0.8. However, the data indicated a tendency for the judges to lower the flavor score of milk as a result of lipase action even before the nature of the defect was recognized. Samples with an acid degree above 1.4 were graded in the low-fair and poor class.

The studies of Krukovsky and Herrington (7) were concerned with normal milk and do not necessarily apply to homogenized milk in which lipolysis is accelerated. This is indicated by the results of Gould and Trout (2) which showed homogenization to result immediately in greatly increased acid degrees in the fat, and also, by the observations of Trout and Scheid (9) that certain commercial plants homogenized raw milk and then pasteurized it immediately without any detectable flavor change. Trout and Scheid also demonstrated the feasibility of this practice. Lane and Hammer (8) observed the flavor of cheese made from a mixture of raw skimmilk and raw homogenized cream to be good even though the fat acidity was high (acid degree 9.7 in one case, 8.2 in another). However, flavor production in cheese is not necessarily analogous to that of milk.

The experiments herein reported were conducted with the view of securing information on the relationship between the acidity of fat from homogenized raw milk and the rancid flavor. The problem was investigated from two angles: (a) by homogenizing raw milk and then making fat and flavor examinations at different intervals, (b) by homogenizing fat possessing different acid degrees and secured from non-homogenized and homogenized raw milk into pasteurized skim milk and then examining the mixture for the rancid flavor.

EXPERIMENTAL PROCEDURE

Milk used in these studies was selected from milk delivered to the College Creamery, care being used to obtain milk practically free of off-flavors. Homogenization of this milk was at 700 pounds pressure and at 37° C. Fat

was secured for analysis by separation, churning and filtering. Ten gram samples were titrated for free fatty acids with 0.05 N NaOH. The free fatty acid content is expressed as acid degree (mls. of N NaOH per 100 gms. of fat).

EXPERIMENTAL RESULTS

Homogenized raw milk. In this phase of the study, selected raw milk was standardized to approximately 10 per cent fat by separation and this milk then divided into two portions. One portion served as the control and was non-homogenized, the other portion was homogenized at 700 pounds pressure and then stored at approximately 25° C. A sample of the homogenized milk was secured immediately after homogenization and at intervals thereafter (usually 15-minute intervals) until the milk had become definitely rancid. At specified intervals, samples, including the control, were

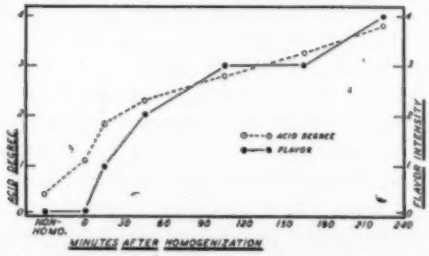

FIG. 1. Relationship between intensity of rancid flavor and increases in acid degree following homogenization of the milk at 700 pounds pressure.

quickly heated to 142–144° F. for 20 minutes and then cooled. After samples for the trial were obtained, portions of each were examined "blind" organoleptically, and other portions were used to supply the fat for titration. Seventeen trials were conducted and six to nine samples were examined for changes in flavor and fat acidity in each trial.

Average results of four trials in which the samples were secured at the same intervals before and following homogenization are illustrated in figure 1. This figure portrays (a) the general relationship between increase in acid degree and development of rancidity; (b) the fact that considerable increase in acid degree may occur before the rancid flavor is detectable but that rancidity develops within a few minutes after homogenization.

Homogenization increased the acid degree of the fat approximately threefold, *i.e.*, from 0.43 to 1.2, without producing a rancid flavor that was detectable by taste. Within 15 minutes the acid degree had increased to approximately 1.8 and the judges usually graded the flavor as "questionable" in regard to the rancid flavor. By the end of 30-minute and 45-minute periods the samples were usually rancid and the acid degree of the fat for the 45-minute period averaged approximately 2.3.

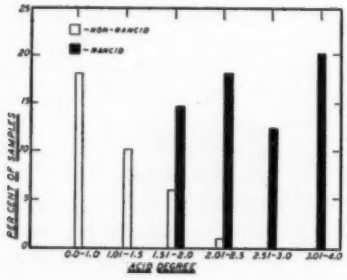

FIG. 2. Distribution of non-rancid and rancid samples of homogenized milk on basis of acid degree of the fat.

The relationship between acid degree and rancidity of all of the samples examined in the 17 trials is illustrated by figure 2. This graph reveals that the rancid flavor was generally detectable when the acid degree was within the range of 1.5 to 2.0. Below an acid degree of 1.5 no samples were criticized as being rancid whereas practically the reverse condition held when the acid degree was above 2.0. Of all the samples examined, approximately one-third were adjudged "non-rancid," with 28 per cent having acid values of 1.5 or less. Only one sample having an acid degree above 2.0 was graded non-rancid.

Fat from non-homogenized and homogenized raw milk: In the second phase of this portion of the study, fat of varying acidities was secured from non-homogenized and homogenized milk. This fat was then re-homogenized into pasteurized skimmilk in proportions to give a 5 per cent milk and the mixture examined organoleptically. Results of several typical trials are presented in table 1.

These data reveal that in no case was a rancid flavor produced in the fat-skimmilk mixture even though the acid degree of the fat ranged from 0.5 to 11.5. Instead, an oxidized, oily, or tallowy flavor resulted in the samples; this flaovr appearing in low acid-degree samples as well as in the high, but to

TABLE 1

Effect on the flavor of the mixture when butter fat of varying acid degrees is homogenized into skim milk

	Trial 1		Trial 2		Trial 3	
	Acid degree of fat	Flavor of mixture	Acid degree of fat	Flavor of mixture	Acid degree of fat	Flavor of mixture
Skimmilk	.....	OK	.....	OK	.....	OK
Skimmilk plus fat	0.5	Oxidized	0.5	Oxidized	0.5	Oxidized
Skimmilk plus fat	1.5	Oxidized	1.5	Oxidized	6.5	Oxidized
Skimmilk plus fat	2.5	Oxidized	2.5	Oxidized	0.6	Oxidized
Skimmilk plus fat	3.5	Oxidized	4.0	Oxidized	7.5	Oxidized
Skimmilk plus fat	4.3	Oxidized	.....	.....	0.5	Oxidized
Skimmilk plus fat	5.6	Oxidized	.....	.....	11.5	Oxidized

slightly less intensity. The degree of oxidized flavor was never sufficient to have obscured the rancid flavor.

Examination of the butteroil used in these trials for the rancid flavor also gave negative results, the oil possessing an oxidized flavor when the acid degree was high. However, when the acid degree was less than 1.0, the butteroil did not uniformly exhibit the oxidized flavor. Although the fat itself, following the removal of the curd and water by filtration, did not possess the rancid flavor, the buttermilk obtained by the churning of cream with a high acid degree was always intensely rancid.

DISCUSSION

The direct relationship between the development of rancidity in milk and the increase in acid degree of the fat as observed in this experiment is in agreement with the findings of Krukovsky and Herrington (7). However, the threshold value for the flavor is approximately twice as high in these studies on homogenized milk as was reported by Krukovsky and Herrington for normal milk. This difference may be explainable on the basis that when lipolysis is accelerated by homogenization, lipase activity proceeds at the expense of those fatty acids which contribute to the titratable acidity of the fat at a proportionately greater rate than is true in the case of non-homogenized milk.

The results secured in this study serve as a basis for explaining the differences which have been reported relative to the correlation between the acid degree of the fat and the rancid flavor. The lack of such a correlation in commercial butter observed by Fouts (1) is understandable in view of the fact that the rancid flavoring materials in the milk or cream are largely lost in the buttermilk when the fat is obtained by churning, even though the fat itself shows the presence of appreciable quantities of free fatty acids. Whether or not butter from rancid cream will retain the rancid flavor would appear to be dependent upon the amount of buttermilk incorporated into the butter, and thorough washing of the butter may completely wash away the rancid-flavored fatty acids. The author has observed that the churning of rancid cream did not uniformly result in rancid butter. This does not eliminate the possibility, however, of the butter becoming rancid on storage due to continued lipase activity. The problem of water solubility of the fatty acids responsible for the rancid flavor is considered more thoroughly in the subsequent paper.

The fact that samples of butteroil with high acid degree produced an oxidized or oily flavor when homogenized in skimmilk does not necessarily mean that there is a relationship between free fatty acid content and the oxidized flavor under these conditions. Such a relationship was not apparent since fat of low acid degree also produced this flavor in the milk. The important point is that the rancid flavor was not produced by this process, even though the fat was obtained from rancid homogenized raw milk.

The apparent absence of at least a portion of the lower fatty acids from butteroil prepared by churning the cream and filtering the fat as indicated by the absence of the rancid flavor may tend to cast some doubt as to the reliability of the titration of the fat as a means of measuring lipolysis. It does appear that such titration values do not necessarily represent all of the fatty acids that are freed by lipase activity, although the results of Gould and Trout (2) would indicate, at least, almost complete measurement of these acids. To secure greater accuracy it may be desirable to utilize some other method than churning in obtaining the fat from the milk or cream. The author has made use of an extraction procedure and the results secured were encouraging. Higher fat titers were secured in the extracted fat than in the churned fat, but the possibility that these higher values may be due to the inclusion of other than fatty acids has not been eliminated.

CONCLUSIONS

Raw milk, homogenized at 700 pounds pressure, is usually rancid when the acid degree of the fat is within the range of 1.5 or 2.0.

Fat secured from rancid milk and with acid degrees as high as 11.5 did not itself possess a rancid flavor nor did it produce a rancid flavor when homogenized into pasteurized skimmilk.

Free fatty acids in butterfat which is obtained by churning are not responsible for the typical rancid flavor of dairy products.

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SOLUBILITY AND VOLATILITY OF FATTY ACIDS INVOLVED IN LIPOLYSIS IN HOMOGENIZED RAW MILK*

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Homogenization of raw milk, a process known to greatly accelerate lipase activity, results in immediate increase in the free fatty acid content of the fat with homogenization at 1500 pounds pressure increasing the free fatty acids approximately five-fold within a few minutes following processing (6). Since information relative to the characteristics of the fatty acids involved in this lipolysis is meager, experiments were conducted to ascertain their solubility and volatility. The results of these experiments are herein presented.

Steam distillation procedures have been used to study the volatility of free fatty acids in milk and milk products. Roahen and Sommer (9) used this procedure to measure the extent of lipolysis in milk and cream and Fouts (4) studied the volatility of free fatty acids in butteroil of varying acid degrees secured from butter by steam distillation of 10 grams of fat and collecting and titrating 200 ml. of distillate. He secured values for volatile fatty acids approximating 15 per cent of the total fat acidity. Davies (2) reports that volatile acids account for less than five per cent of the total titratable value of the fatty acids, whereas oleic acid is responsible for 60–70 per cent of this value.

Dyer (3), and Hiscox and Harrison (7), used steam distillation in the study of pure volatile organic acids. Hiscox and Harrison (7) steam distilled acetic, butyric, caproic, caprylic, capric, and lauric acids (acids neutralized with NaOH and then acidified to pH 2 with H_2SO_4) and secured complete recovery from water when distillate volume was "5 times original volume." Recovery of these acids from cheese was retarded, the retardation being especially marked for acetic, caprylic, caproic, and lauric acids. Butterfat and cheese fat also retarded distillation of the water-insoluble fat-soluble acids.

Methods of preparing samples of cheese for steam distillation were studied by Hiscox, Harrison, and Wolf (8). They found that a method involving warm water washing of the cheese to obtain the fat, ether extraction of the fat, washing of the fat with NaOH, acidifying and steam-distilling this rinse, resulted in volatile acid values which were about 4.5 times as high as those from direct steam distillation in the case of Stilton cheese and about twice as high in the case of Cheddar.

Received for publication July 24, 1943.

* Journal Article No. 657, new series, Michigan Agricultural Experiment Station.

PROCEDURE

The studies reported in this paper were conducted with two purposes in mind: (a) to determine the solubility of fatty acids in fat from homogenized raw milk by washing the fat either with water or with a weak alkaline solution; (b) to determine the volatile fatty acid content of the butterfat by direct steam distillation of the butterfat itself and by the distillation of the alkaline rinsings of the butterfat.

Butterfat with high free fatty acid content was produced by homogenization of raw milk at 37° C. and at 500 pounds pressure. The butterfat was secured by churning, melting, centrifuging, and filtering.

Fat acidity was determined by the official procedure (1) with exception that a 10-gram sample, 25 ml. ethyl alcohol, and N/20 NaOH solutions were used. The free fatty acid content is expressed as acid degree (mls. of N NaOH per 100 gms. of fat).

A multiple steam distillation apparatus, capable of distilling four samples at once, was utilized for the studies on volatile acids. This apparatus consisted of a sodium chloride bath maintained at a temperature of 102–104° C. The bath was of sufficient size to accommodate four one-liter round bottom flasks containing the samples. Steam was generated in two five-liter round bottom flasks, each flask supplying steam for two samples. The steam entered the sample through Folin-type ammonia tubes with perforated bulbs at the bottom, and this inlet tube extended well below the surface of the samples. A Kjeldahl connecting bulb was placed at the outlet from the flask to insure against entrainment of the sample. Condensation of the steam enroute to and from the sample flask was reduced to a minimum by wrapping the conducting glass tubes with asbestos. By regulation of the temperature of the bath and the rate of steam it was possible to maintain fairly constant volume in the flasks containing the samples.

The volatile material leaving each sample passed to a Liebig condenser where it was condensed. The distillate then passed through a No. 40 Whatman filter paper to remove the water insoluble acids and into a 200-ml. volumetric flask. Titrations were conducted in each 200 ml. sample with 0.05 N NaOH, using phenolphthalein as the indicator. After each 200 mls. of distillate were secured, the condensers and the filters were washed with ethyl alcohol to dissolve the alcohol-soluble fatty acids. This alcohol fraction was titrated and the titer added to the values secured for the 200 mls. of distillate.

Blank distillations were made on water at pH 2. The blank values, which included those for the alcohol used to rinse down the condensers, averaged approximately 0.35 ml. of 0.05 N NaOH per 200 mls. of distillate.

RESULTS

Solubility of free fatty acids. In other work (5) the senior author observed that butterfat, secured from rancid cream by churning and filtering,

and with a high free fatty acid content did not possess a rancid flavor. This observation raised the question as to the water solubility of these fatty acids and trials were conducted to ascertain their solubility by washing the butterfat with warm water. In this experiment, approximately 20 gms. of butterfat having a high content of free fatty acids (acid degree approximately 5) were placed in a separatory funnel and shaken for 30 seconds with 20 ml. of water at 130–135° F. The fat layer was then permitted to rise and the aqueous layer removed and titrated. This washing was repeated six times. After the final washing, the fat was removed, dried, and titrated. Results of the original and final titrations on the fat are shown in table 1.

These results show no decrease in the acid degree of the fat to occur with six washings with warm water. Although the data are not shown, titrations of the washings revealed no measurable quantity of fatty acids were contained therein. These findings indicate either that the water-soluble fatty acids are not retained by the fat during its churning and purification or that they have greater affinity for the fat than for the water.

TABLE 1

Influence of washing butterfat with water on the acid degree

	Trial 1	Trial 2	Trial 3	Trial 4
Original acid degree of fat	4.93	4.87	4.82	4.80
Final acid* degree of fat	4.91	4.88	4.85	4.85

* Following washing of 20 grams of fat with six successive portions of warm water.

Other trials were conducted to determine the efficiency with which a weak alkaline solution may remove the fatty acids from fat. The method used was similar to that proposed by Hiscox *et al.* (6) for cheese. Fat samples weighing either 25 or 50 grams were dissolved in 100–120 mls. ethyl ether in a separatory funnel. Twenty-ml. portions of 0.05 N NaOH were added and the mixture shaken gently with rotary motion to avoid emulsification. The aqueous layer was permitted to settle and was drained away. The fat-ether mixture was then washed with 20-ml. portions of distilled water, the ether evaporated on a hot plate and in a 100° C. oven, and the fat titrated for acidity. Results are presented in table 2.

These data reveal that this procedure of removing fatty acid is highly efficient, with three 20-ml. portions of 0.05 N NaOH being sufficient to reduce the fat titer by more than 90 per cent in a 50-gram sample when the acid degree approximates 3 to 5. However, there appears to be a point of irreducible minimum in the fat acidity and additional washings do not appreciably alter this value. This residual acidity amounts to approximately 0.15–0.30 ml. of 0.05 N NaOH per 10-gm. sample of fat. These values may be due to a slight degree of hydrolysis of the fat or a slight extent of endpoint fading occurring during the titration procedure. On the basis of

these results it would appear that three to five washings of fat with small portions of weak alkaline solutions, followed by two rinsings with water, will remove practically all of the free fatty acids, provided that the alkali used is in excess to that required for neutralization by at least 75 per cent. This amount of washing is decidedly less than was used by Hiscox, Harrison, and Wolf (7) for cheese fat.

Steam distillation of fatty acids. Steam distillation of fatty acids was conducted using the multiple unit described under "Procedure." In preliminary trials, butyric acid (Eastman) was used for the distillation. Suffi-

TABLE 2

Efficiency of removing fatty acids from a fat-ether mixture by washing with a weak NaOH solution

Trial No.	Size of sample	Amount of ether	Amount of*		Titer of fat†			Volume alkali times volume of fat	Excess of 0.05 N NaOH solution
			Washing with 0.05 N NaOH	Rinsing with water	Original	Final	Reduction		
	gms.	*ml.*	*ml.*	*ml.*			%		*ml.*
1	50	120	2–20	2–20	7.47	0.83	88.88	0.8	2.65
2	50	120	3–20	2–20	7.46	0.45	93.97	1.2	22.70
3	50	120	4–20	2–20	7.46	0.30	95.98	1.6	42.70
4	50	120	4–20	2–20	6.90	0.23	96.67	1.6	45.50
5	50	120	5–20	2–20	6.90	0.25	96.38	2.0	65.50
6	50	120	5–20	1–20, 2–10	6.90	0.15	97.83	2.0	65.50
7	50	120	6–20	2–20	6.90	0.30	95.65	2.4	85.50
8	50	120	6–20	2–20	6.90	0.20	97.10	2.4	85.50
9	50	120	7–20	2–20	8.60	0.20	97.68	2.8	97.00
10	25	100	8–20	2–20	8.45	0.23	97.28	6.4	138.87
11	25	100	4–20	4–20	8.45	0.20	97.63	3.2	58.87
12	25	120	5–20	4–20	8.45	0.18	97.87	4.0	78.87
13	25	120	4–10	4–10	8.45	0.23	*97.28	1.6	18.87
14	25	120	5–10	4–10	8.53	0.25	97.07	2.0	28.67

* First values indicate number of washings, second values indicate the volume used per washing; thus 2–20 indicates 2 washings of 20 ml. each.

† Titer of fat represents mls. 0.05 N NaOH solution per 10 gms. To change to acid degree divide by 2.

cient butyric acid was used to require 20–25 ml. of 0.05 N NaOH for neutralization. This acid was neutralized with the alkali, then adjusted to pH 2 with H_2SO_4. The volume was standardized to 200 ml. and steam distillation was conducted. Each 100 ml. of distillate was titrated and corrected for the blank value. Average results secured for seven trials are presented in figure 1, in which a comparison is made with the results of Hiscox and Harrison (7). The two curves exhibit similar trends and indicate that when the volume of distillate is twice the original volume 98 per cent of the acid is recovered. These data also are in agreement with those secured by Dyer (3). Although results are not presented, distillation of n-caprylic acid revealed even more rapid recovery, with approximately 93 per cent being recovered

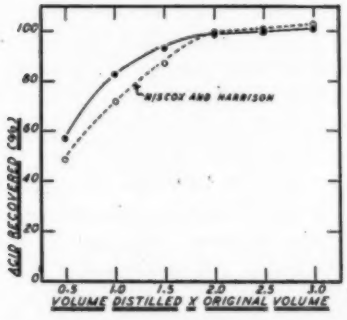

FIG. 1. Rate of steam distillation of n-butyric acid in comparison to rate observed by Hiscox and Harrison.

with distillation of one times the original volume. This compares favorably with the 95 per cent reported by Hiscox and Harrison (7).

Following the standardization of the distillation procedure by use of the pure fatty acids, trials were conducted in which butterfat having high free fatty acid content was steam distilled. In some trials, approximately 150 grams of butteroil were used, in other trials, the size of the sample was approximately 50 grams. The volume of the sample was increased to 200 ml. with acidified water and 200 ml. portions of the distillate were titrated. Results of 7 trials are presented in table 3.

Data in this table reveal that steam distillation of the pure butteroil results in poor recovery of the fatty acids. This may result due to the fact that no appreciable quantity of the lower volatile fatty acids are in the free state, or to the retardation action of the fat. The volatility of 4–6 per cent of the fatty acids is considerably lower than 15 per cent reported by Fouts (4). However, Fouts titrated 200 ml. of distillate from 10 grams of fat, a value representing 20 times the original volume as contrasted to 6 to 10 times the original volume used in the experiments herein reported.

TABLE 3

Amount of volatile fatty acids recovered by steam distillation of butterfat with high free fatty acid content

Sample No.	Acid degree of fat	Per cent of total free fatty acid after distilling		
		3 × original vol.	6 × original vol.	10 × original vol.
1	3.62	2.43	5.03	.....
2	4.25	2.10	3.14	4.02
3	4.25	2.28	3.38	4.34
4	4.35	1.84	2.73	3.96
5	4.33	2.35	3.34	4.50
6	6.75	2.35	5.62	.....
7	7.00	2.86	6.12	.....

To study further the volatility of free fatty acids in butterfat, butterfat having previously undergone considerably lipolysis was divided into two lots. One lot was steam-distilled directly. The other lot was dissolved in ether, washed with 0.05 N NaOH solution and then rinsed with distilled water to remove the remainder of the salts. Fifty grams of fat were used, washed with six 20-ml. portions of the alkali, and rinsed with two 20-ml. portions of water. The alkaline washings and the water rinse were combined, adjusted to pH 2 with H_2SO_4 and the solution distilled. Two-hundred-ml. distillate portions obtained from the fat and from the alkaline rinsings of the fat were titrated. Acid degrees of the fat used in this study ranged from 3.62 to 7.0. Average results are portrayed in figure 2.

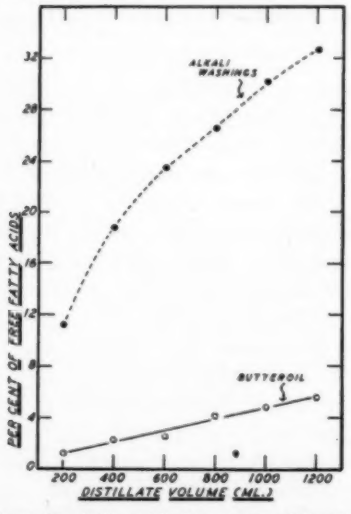

FIG. 2. Recovery of volatile fatty acids from butteroil having high acid degree when steam distillation was conducted on the butteroil and on the alkali washings from the butteroil. (Original volume—200 ml.)

This figure illustrates the remarkable difference in volatility of fatty acid when the acids are removed from the fat and then distilled and when they are distilled directly from the fat. Distillation of six times the original volume resulted in a recovery of only 5.7 per cent of the acids when the distillation involved the fat itself, whereas a recovery of 32.7 per cent resulted when the fatty acids were distilled separately. The rate of distillation of the fatty acids from the acidified-alkaline rinse is also interesting. Approximately 30 per cent of the fatty acids recovered during the total distillation period were recovered by distillation of one times the original volume. However, an appreciable quantity of acids were being distilled at the close of the distillation.

Results secured with the acidified-alkaline rinse indicate that a higher percentage recovery of fatty acids may be obtained from lower acid degree fat. In one trial in which the fat possessed an acid degree of 3.62, 48.8 per cent of the acids were recovered by distillation of seven times the volume, whereas under similar conditions, fat with an acid degree of 7 permitted a recovery of 31.4 per cent. However, additional information is needed in this connection before conclusions are drawn.

DISCUSSION

The failure of the washing of the fat with successive portions of warm water to reduce the fatty acid content indicates the possibility that water-soluble fatty acids either are not retained in any appreciable quantity by the fat or are held in such a manner that they are not removed by the water-washing procedure. These findings demonstrate that washing of the butter granules following churning and during the preparation of butteroil for fat titrations will likely have no influence on the resulting titer of the fat. Thus, the preparation of butteroil in lipolytic studies may be greatly expedited by utilizing water to remove the milk-solids-not-fat.

The steam-distillation studies show the undesirability of attempting to measure lipase activity in the milk or cream by steam-distillation of the fat. The proportion of volatile acids obtained by this procedure is so small that differences between samples may not be clearly demonstrated. In contrast, the steam-distillation of acidified-alkaline washings of fat appears to be an excellent method of studying lipolysis. Recovery of the acids was approximately five times as great for the alkaline washings as for the butteroil when the volume of the distillate was six times the original volume.

The high proportion of volatile acids secured when the acids were distilled separately from the fat may serve to indicate that the fatty acids involved are of the water-insoluble fat-soluble type. Hiscox and Harrison (7) found butterfat to distinctly retard the distillation of this type of acid, with the retardation increasing with increasing solubility of the acids in fat. This fat retention effect is especially marked in the results presented herein. In addition, the fact that these acids are not removed by washing fat with water and that the rancid flavor is not retained by the fat also indicates that the free fatty acids present in churned fat are of the fat-soluble type.

CONCLUSIONS

The fat acidity of butterfat from homogenized raw milk was not appreciably affected by washing with six successive portions of warm water. However, washing of an ether solution of the fat with a weak alkaline solution is an efficient method of removing the free fatty acids.

Steam distillation of butterfat is highly inefficient as a means of recovering volatile fatty acids, resulting in a recovery of approximately 5 per cent

when distillate volume was six times original volume. In contrast, distillation of acidified-alkaline washings of fat resulted in a recovery of approximately 32 per cent of the free fatty acids.

Free fatty acids in butterfat secured from homogenized raw milk by churning are apparently of the water-insoluble fat-soluble type.

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THE INFLUENCE OF LOW ENVIRONMENTAL TEMPERATURES ON INTRAMAMMARY TEMPERATURES*

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Since temperature plays an important part in all physiological processes it is only consistent to expect that variations in the temperature of the udder should affect the production of milk. Although the rectal temperature of the normal cow lies between 100° and 103° F., one would expect the udder, with its comparatively large exposed surface, to be influenced to some extent by the environmental temperature. Many reports have been published dealing with the effect on milk production of low environmental temperatures but no information regarding intramammary temperatures has been found.

Since the teats and lower portion of the udder are more exposed than other portions, one would expect temperature changes in this area to be most marked. Therefore, for the most part, only data on the temperature in the teat and the milk cistern have been used in this study.

Two methods of obtaining the intramammary temperature were used. Inserting a small metal thermometer (Model 226L001, Weston Electric Instrument Company) directly into the teat cistern and making readings as soon as a constant temperature was indicated, was one. Hereafter, this method will be designated as the "direct method." The other method, an "indirect" one, was that of estimating the intramammary temperatures from the temperatures of the first drawn milk.

The direct method of obtaining the intramammary temperature is unquestionably the better method. However, due to the danger of introducing infection into the udder, only cows which were to be discarded for milk purposes could be used in this manner. Of the eight cows on which direct temperature readings were obtained, four were reported by our veterinary department to be mastitic in one or more quarters. However, none of these cows had acute mastitis in the commonly accepted meaning of the term.

Cows with mastitis were kept in a separate barn from the one housing the healthy animals. The removal from the herd at frequent intervals of all cows infected with mastitis as well as the difficulty of carrying out even two trials at the same environmental temperature made it impossible to secure adequate data by the direct method. For these reasons it seemed wise to check the direct method by an indirect method and then use the indirect method to obtain more data from cows free of mastitis.

Received for publication July 26, 1943.

* Journal Paper No. J–1137 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 814.

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During the exposure period, the cows were confined to a small protected yard just south of the barn. Some of the differences noted with the same cow and the same environmental temperature may have been due to differences in activity of the cows from day to day and to wind velocity. Also, when the sun was shining, the environmental temperature tended to fluctuate rapidly. Data showing the effect of repeated sampling, *et cetera*, were obtained in the barn proper after the severe winter weather had passed.

Before securing direct readings each cow was brought into a room whose temperature varied from 65° to 75° F. The udder was washed and disinfected. The thermometer whose stem was seven and three-fourths inches long, was inserted as far as possible into the mammary gland and readings made by means of a mirror held beneath the inverted dial. The dials were graduated in two degree intervals and the thermometers were guaranteed by the makers to be accurate to within one-half of one per cent, which was verified. The order in which readings were taken is indicated in the tables.

In making indirect readings, the udders were quickly washed with chlorine solution and samples collected as soon afterwards as possible. The samples of milk were collected in four test tubes, one for each quarter of the udder. These 60-ml. test tubes were each inserted into pint thermos bottles and securely attached so as to be doubly insulated from the effects of the temperature of the room in which readings were made. Cork stoppers with small thermometers (like those commonly used in the Van Slyke blood gas apparatus) completed the assembly.

Just before milk samples were obtained the thermos bottles were removed from the constant temperature oven and brought to the room where temperature readings were made. Each 60-ml. tube was emptied of water, filled at once with warm milk, preferably from a cow subjected to the same temperature changes as the environmental animals, and the tubes corked. In taking milk samples from experimental animals, the "tempering milk" was discarded and each tube filled at once with milk from a quarter of the udder of an experimental animal. The thermos bottles were held close to the end of the teat and were filled with milk as rapidly as possible. Due to foaming only about 50 ml. of milk were removed at each sampling. The tubes were then corked and the thermos bottles inverted so that milk and not foam was sure to cover the thermometer bulbs. Readings were made as soon as samples from all quarters had been obtained.

RESULTS

Before attempting to study the effect of low environmental temperatures on the udder of the cow it seemed wise to determine the normal range of udder temperatures. Table 1 not only shows the udder temperatures by the direct and indirect method, but data are also presented on temperatures of each quarter both for healthy cows and those considered to be carriers of

mastitis. These values were obtained after the cows had been in the barn for several hours. Although barn temperatures varied from 58° to 70° F. at different times during the experiment, readings were obtained for all quarters of the udder at any one temperature.

With some cows the temperature of the milk in the quarter sampled last was higher than the milk in the quarter sampled first. This was not consistently true. There was a definite tendency, though, for the milk in the two front quarters to be slightly higher in temperature than the milk in the two rear quarters. There was no regular difference between the intramammary temperatures of cows free of mastitis and those known to have been previously infected with mastitis.

TABLE 1

Data showing average intramammary temperature of udder with environmental temperatures of 58° to 70° F.

Quarters (observations made in order indicated)	No. of observations	Direct method*	No. of observations	Indirect method†
Cows free of mastitis				
		°F.		°F.
1. Right front	10	99.1	28	97.9
2. Right rear	10	98.7	28	97.6
3. Left rear	10	98.7	28	97.4
4. Left front	10	99.1	28	97.8
Two front	....	99.1	....	97.85
Two rear	....	98.7	....	97.5
Cows with mastitis				
1. Right front	31	99.0	30	97.6
2. Right rear	31	98.7	30	97.9
3. Left rear	31	98.5	30	97.2
4. Left front	31	99.0	30	97.6
Two front	....	99.0	....	97.6
Two rear	....	98.6	....	97.5

* Thermometer inserted into gland cistern.
† Temperature of first drawn milk.

The intramammary temperatures secured by the indirect method are about 1.2 degrees lower than those obtained by the direct method. These differences were greater when barn temperatures were low and with cows which milked slowly. The intramammary temperature, as obtained by the direct method, showed no consistent change with the usual fluctuations in barn temperature (58° to 70° F.), so it is apparent that the indirect method becomes less reliable as lower environmental temperatures are studied.

Variations of the intramammary temperature for the same cow varied between trials as much as 1° F. by the direct method and 1.5° F. by the indirect method. Changes in environmental and body temperature caused some of this fluctuation. Washing the udder before collecting the samples

may have produced different responses on different days. Differences in the amount of bedding protecting the udder from the barn floor also varied from day to day and may have caused some minor fluctuations in intramammary temperature.

Although more pendulous udders might be expected to show lower temperatures than the so-called "tight vessel" type, no definite trend was evident at barn temperatures.

The data presented in table 1 have been rearranged in table 2 so as to show whether the breed or the milk volume of the udder influenced the intramammary temperature. No evidence was found to indicate that either factor is important when the environmental temperature is between 58° and 70° F.

TABLE 2

Effect of milk production and breed on intramammary temperature (indirect method)

No. and breed of cows in each trial	No. trials	Average daily production			
		> 60 lbs.	25–60 lbs.	< 25 lbs.	All cows
		Temperature*	Temperature*	Temperature*	Temperature*
		°F.	°F.	°F.	°F.
20 Holstein	3	98.2	97.9	.....	98.0
6 Guernsey	3	.....	98.0	98.3	98.1

* Temperature of first drawn milk. Environmental temperature (barn temperature) varied slightly but samples from every cow were taken at each trial.

TABLE 3

*Temperatures of consecutive samples of milk obtained from the same udder**

Cows used in each trial	Trials	Average temperature of milk from all quarters		
		First sample	Second sample	Third sample
No.	*No.*	°F.	°F.	°F.
20	3	98.9	98.9	99.1
3	1	98.1	98.7	99.2

* Data obtained during each trial were taken at the same environmental temperature. The environmental temperature between trials varied from 58–68° F.

In trials, results of which are reported in table 3, a second series of milk samples was taken as soon as the temperatures on the first series of samples had been noted. And then a third series of samples was taken from the same cow, all within about a three-minute interval. During this interval most of the cows had begun to "let down" their milk. Although the last series of samples gave higher values than the first two, the temperature differences were not very important.

Only a very small amount of data bearing on the importance of this "letting down" process were obtained with cows exposed to low environmental temperatures. By the indirect method, readings were obtained on four cows after three hours' exposure at 20° F. (table 4). The udders of

TABLE 4

Effect of handling the udder on the temperature of the first drawn milk obtained from cows exposed to an environmental temperature of 20° F. for 3 hours

	Front quarters in °F.	Rear quarters in °F.	All quarters in °F.
Cow No. 1701			
Udder not handled before sample was taken	95.9	95.4	95.6
Cow No. 1044			
Udder washed before sample was taken	97.2	96.8	97.0
Cow No. 1751			
Udder not handled before sample was taken	94.1	92.6	93.7
Cow No. 1756			
Udder washed before sample was taken	97.4	96.7	97.0

two of these cows were washed and disinfected as already noted in the methods of procedure. With the other two cows, milk samples were withdrawn without previous handling of the udder. Although there were always differences in intramammary temperature between cows exposed to the same environmental temperature, the striking differences noted in table 4 would indicate that the handling of the udder is likely to cause the cows to "let down" their milk and that this phenomenon explains why the milk in the udders of these cows tends to "heat up," probably by adding more milk from higher up in the gland to that already present in the milk cistern.

TABLE 5

Relation between rectal temperature and intramammary temperature when the environmental temperature varied from 58° to 70° F.

Rectal temperature in ° F.†	No. of cows	Total No. of observations	Average intramammary temperature*		
			Fore quarters in °F.	Rear quarters in °F.	All quarters in °F.
100.8	4	24	99.1	98.9	99.0
101.0	3	9	98.7	98.7	98.7
101.1	1	1	98.7	98.8	98.7
101.2	5	25	98.5	98.6	98.6
101.3	3	6	99.2	98.9	99.0
101.4	5	30	99.0	98.8	98.9
101.5	3	12	98.0	97.2	97.6
101.6	4	12	97.8	97.6	97.7
101.7	2	2	97.7	97.3	97.5
101.8	2	4	98.3	98.3	97.3
101.9	3	6	97.7	97.3	97.5
102.0	2	4	98.1	97.9	98.0
102.1	1	1	97.7	97.3	97.5
102.2	1	1	99.4	99.2	99.3
103.1	1	1	99.4	100.0	99.7
103.2	1	1	100.4	100.3	100.4

* Indirect method.

† Rectal thermometer with extended shank was used so that bulb was inserted two inches deeper than normally.

TABLE 6

Relation between environmental temperature and intramammary temperature

Environmental temperature in °F.	No. of cows	Total No. of trials	Temperature in °F.			
			Fore quarters	Rear quarters	Average	Rectal
Direct method						
−14	2	1	97.1	96.6	96.9	101.5
−12	1	1	96.2	96.0	96.1	101.4
−10	2	1	95.8	95.3	95.5	101.0
9	2	1	98.2	97.2	97.7	101.3
14	2	2	97.9	96.9	97.3	101.7
16	2	1	98.3	98.0	98.1	101.9
17	2	1	98.3	97.6	97.8	101.6
18	1	2	98.2	97.7	97.9	102.0
19	1	1	98.6	97.7	98.1	101.9
20	2	3	98.0	97.6	97.8	101.7
28	3	2	98.6	97.9	98.2	101.5
29	3	1	98.8	98.8	98.8	101.5
30	2	1	98.7	98.1	98.4	101.6
31	5	1	98.7	97.8	98.2	101.7
32	1	1	98.7	98.3	98.5	101.8
40	1	2	99.0	98.9	98.9	101.7
44	3	1	98.6	97.0	98.1	101.9
48	3	1	98.5	98.3	98.4	101.7
49	2	1	99.2	98.7	98.9	101.3
52	1	2	99.3	99.0	99.1	101.9
54	2	1	99.1	98.7	98.9	101.6
55	2	1	99.0	98.6	98.8	101.4
56	1	1	99.3	99.0	99.1	101.3
58	3	1	99.0	98.6	98.8	101.9
60	2	1	98.8	98.5	98.7	101.6
61	2	1	99.0	98.9	99.0	101.5
65	2	1	99.0	98.5	98.8	101.4
Indirect method						
14	1	1	98.3	97.3	97.8	101.1
16	4	1	96.7	97.6	96.2	101.5
17	3	1	96.4	95.7	96.0	101.6
18	6	1	96.2	95.4	95.8	101.9
19	3	1	96.9	95.8	96.2	101.3
20	4	3	96.7	95.5	96.1	100.9
28	5	2	97.6	97.1	97.3	101.5
29	3	1	97.7	97.5	97.7	101.5
30	4	2	97.7	97.1	96.3	102.1
31	2	1	97.1	96.7	96.9	101.8
32	3	2	97.1	96.3	96.7	101.8
35	5	1	97.5	97.3	97.4	101.4
40	4	2	97.7	97.1	97.4	101.7
48	1	1	97.2	97.0	97.1	101.9
49	1	1	97.9	97.3	97.6	101.6
52	4	2	97.5	97.0	97.2	101.7
56	3	1	97.5	97.2	97.3	101.8
57	6	1	98.8	98.7	98.7	101.3
58	14	1	99.0	98.9	98.9	101.3
68	9	1	99.1	99.3	99.2	101.2
70	21	1	98.6	98.4	98.5	101.4

Since rectal temperatures were always taken at the same time as the temperatures of the udders were being determined, it seemed wise to see if there was any relationship between them. These data, shown in table 5, indicate no relationship between rectal temperature and udder temperature except when the rectal temperature rose above 103° F. The samples of milk secured from cows with a high rectal temperature showed a slightly higher temperature than milk from the rest of the herd tested.

The average differences between the rectal and udder temperatures during the winter was 3.6° F., by the indirect method, and 2.7° F., by the direct

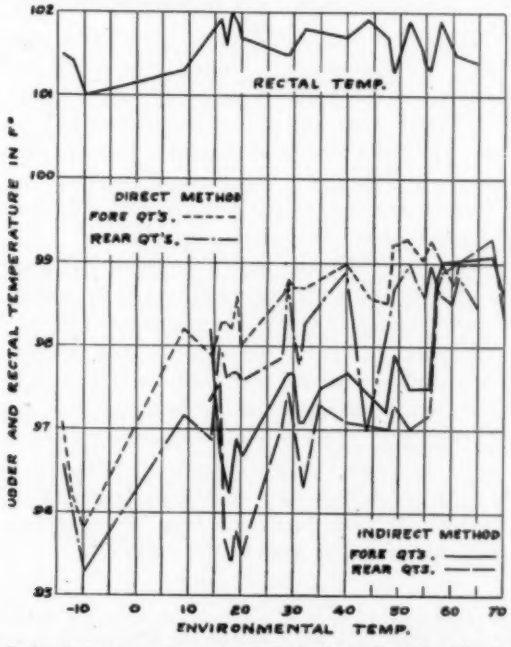

FIG. 1. Relation between environmental, body (rectal) and udder temperatures.

method. The four cows which had a high rectal temperature showed an average difference between rectal and intramammary temperature when measured by the indirect method of only 3.1° F.

Most of the information presented so far was obtained with cows housed in a reasonably warm, well-constructed barn. These data give us some idea of the general range of variability in intramammary temperature which may be expected under a moderate environmental temperature. Data are presented in table 6 which show the effect on the udder of external temperatures ranging from 70° to – 14° F. A total of thirty cows were used in twenty-six trials to obtain this information. Nineteen of these cows were free from mastitis while eleven of them were, or had been, infected with mastitis. The

time of exposure was never less than one hour when direct temperature readings were taken and never less than two hours when indirect readings of udder temperatures were taken. Most of the cows were left outside longer periods unless the weather was very severe.

From the data presented in table 6 and figure 1 it is evident that the intramammary temperature is influenced by low environmental temperatures. During the coldest weather the intramammary temperature was over 5° F. below the rectal temperature when recorded by the direct method. This should be compared with a difference of 2° to 3° F. when the environmental temperature varied between 58° and 70° F. If the cows had been kept quiet and exposed to sub-zero weather, it is quite probable that the difference between the temperature in the teat cistern and in the lower part of the gland cistern, and the rectal temperature, may actually have been as great as 10° F. This is especially true if the animal had lain on the cold ground or snow. The difference in temperature between the fore and rear quarters also became greater as the temperature dropped, probably because of the greater surface exposure of the rear quarters. Differences in rectal temperature as a result of exposure were negligible.

Only one observation was made with a dry cow. This animal was left out of doors for an hour when the temperature was 10° below zero. By the direct method, temperatures of the different quarters were as follows: right front, 96° F., right rear, 93° F., left front, 94° F., and the left rear, 91.9° F. The unusually low readings for the rear quarters may have been caused by obstructions which would not allow the thermometer to be inserted farther than the base of each teat. This cow's udder was very pendulous and the teats large. In spite of this condition she never appeared to suffer from exposure while cows which were milking heavily occasionally had the ends of their teats frosted. In fact, the danger from low temperatures appeared greatest with cows whose udders were full of milk.

CONCLUSIONS

When cows were kept in a barn where the temperature varied between 58° and 70° F., the temperatures of their udders (teats and milk cisterns) averaged 2.5° to 3.0° F. lower than their rectal temperature. When cows were exposed to sub-zero weather the observed differences increased to around 5° F. The movement of the cows into the barn and the washing of the udder before inserting the thermometer into the gland cistern decreased the actual difference between rectal and udder temperatures. Otherwise, greater differences would have been observed.

ACKNOWLEDGMENT

The authors wish to express their appreciation to Dr. B. L. Herrington of the Dairy Industry Department at Cornell University, for suggesting this problem.

THE COMPARATIVE EFFECTS OF CONTINUAL AND ROTATIONAL SYSTEMS OF GRAZING ON THE CAROTENE CONTENT OF PERMANENT PASTURE HERBAGE AND OF THE MILK PRODUCED THEREFROM*

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The favorable returns from intensive systems of pasture management in Europe have attracted widespread attention in this country. One of the foremost advantages affirmed for this system is the improvement of the quality of the herbage. Since improved pastures are generally accepted as a practical solution to many difficult nutritional problems in the livestock industry today, any practical measure that reputedly enhances the nutritive value of the pasture plants merits consideration.

Rotational grazing, an essential phase of intensive pasture management, has been reported to increase the carrying capacity of pastures in several cases (9, 10, 19). Conceivably this increased yield could be related to qualitative changes, particularly in carotene values, of the herbage. Since there is a paucity of information on this subject, the need was deemed adequate to warrant investigation.

Thus a determination of the comparative effects of continual and rotational systems of grazing on the carotene of the herbage, primarily, and of the milk, secondarily, constituted a supplementary phase to a three-year study designed to ascertain the applicability of rotational grazing to improved Bermuda grass pastures.

EXPERIMENTAL

Pastures. The grazing areas were improved pastures, previously described (5). The total area of six acres was divided by permanent fences into three two-acre paddocks. Each year one of these was continually grazed while the remaining two, subdivided by temporary electric fences to form a four-unit system, were rotationally grazed. In the course of the three-year investigation each paddock was grazed continually one season and rotationally two seasons.

The flora consisted primarily of hop clover (*Trifolium procumbens* L.) in early spring and Bermuda grass (*Cynodon dactylon* (L.) Persoon) during the remainder of the grazing season. However, small amounts of other

Received for publication August 2, 1943.

* Technical Publication No. 106. Published with the approval of the Director of the South Carolina Agricultural Experiment Station.

plants including white Dutch clover (*Trifolium repens* L.), bur clover (*Medicago arasica*) and common vetch (*Vicia sativa*) also contributed to the early grazing. In late spring during the brief interim between the maturation of the legumes and the development of Bermuda grass to the grazing stage and in the latter part of summer when droughts occurred, available herbage was sometimes sparse.

Annual applications of a 6–12–6 fertilizer were made at the rate of 400 pounds per acre during either late winter or early spring.

At various times throughout the grazing season, the paddocks were mowed to remove ungrazed clumps of grass and to check the growth of scattered areas of objectionable weeds.

Grazing animals. Lactating cows of the Holstein, the Guernsey and the Jersey breeds were used in comparing continual and rotational grazing. Recommended procedures (1) were followed in the selection, allotment and management of the cows.

The pasture grass was supplemented with a simple low-carotene concentrate mixture, which was fed according to the quantity of herbage available and the amount of milk produced by the individual cows. During periods of scanty grazing, beet pulp also was included in barn feeding.

The frequency of transfer from one unit to another in the rotational system varied with the stage of maturity and the rate of growth of the pasture herbage. Hence, the rotational rate was more rapid in spring than in the latter part of the pasture season. During the annual grazing period, an average of six rotation cycles of 29.5 days each were completed. In a representative cycle each pasture unit was grazed 7.5 days, leaving an interim of 22 days for recovery.

Herbage samples. The samples of pasture herbage were composites representing clippings collected at random over the respective pasture areas. The plants were cut at a height approximating the level to which it was grazed by the cows. In the rotational system a sample was taken from each section immediately before the cattle were released onto the area. In the continual system the first sample was gathered shortly preceding the initial grazing, and subsequent samples were collected at the same time as each alternate sample from the rotational series was clipped. This plan resulted in the collection of approximately two samples from the continually grazed area and four from the rotational sections each month; thus the number of samples from the two areas was in the same ratio as the acreage involved.

Each gross sample of collected grass was chopped sufficiently fine to permit accurate sub-sampling for carotene analysis.

The analytical procedure for the determination of carotene was based on the principles set forth by the Association of Official Agricultural Chemists (2) for the preliminary extraction and the modification described by Hegsted *et al.* (7) for the final extraction. The amount of extracted caro-

tene was calculated from colorimetric measurements, using a standardized solution of potassium dichromate as the reference reagent.

Milk samples. Individual milk samples for carotene analysis were collected from a representative of each of the three aforementioned breeds in the respective grazing groups. These samples were collected bi-weekly during three consecutive periods, pre-grazing, grazing and post-grazing. The minimum length of the first and last periods was one month, during which time the cattle were barn-fed, receiving corn silage as the only roughage. The duration of the grazing period varied from year to year, depending primarily on climatic conditions.

Each milk sample was a composite of aliquot portions taken from each of the three normal milkings during a day. In the period between milkings the collected milk was stored at a temperature of 35° to 40° F. Within three hours after the final collection, the sample was composited and subjected to the initial analytical procedures.

The fat of the milk was determined by the standard Babcock method and the carotene was measured by a procedure developed by one of the authors (Mitchell) in 1936. The available methods at that time required the use of milk fat as the starting material. These procedures necessitated not only the use of large volumes of milk but also the expenditure of much time and labor to prepare the fat. In searching for a suitable procedure the Roese-Gottlieb (2) method of extracting fat from whole milk was adopted as the base from which the following procedure evolved:

A 50-ml. portion of milk is placed into a 250-ml. separatory funnel. Seven ml. of ammonium hydroxide and 50 ml. of ethyl alcohol (aldehyde-free) are added successively. The contents of the funnel are shaken after each addition. Fifty ml. of ethyl ether and 50 ml. of petroleum ether (b.p. 35–60° C.) are then added in the order listed and mixed gently after each addition. Vigorous agitation may result in emulsification and poor separation of the ether layer. The contents of the funnel are allowed to stand 30 minutes for complete separation after which the lower layer is drawn off and the remaining material reextracted with a mixture of 25 ml. of ethyl ether and 25 ml. of Skelly solve B (petroleum ether, b.p. 60–70° C.). All material from the two extractions is placed into a distillation flask and distilled under reduced pressure at a temperature of 70–80° C. to remove the more volatile solvents. Usually 10 to 15 ml. of a solution, mainly Skelly solve, is left in the flask.

This residual material is transferred to an Erlenmeyer flask where it is mixed with 25 ml. of an alcoholic potassium hydroxide solution (60 gm. potassium hydroxide made up to one l. with ethyl alcohol). The fat present is saponified by refluxing 30 minutes, after which the contents of the flask are cooled and transferred to a separatory funnel by rinsing with 20 ml. of water. The material is extracted twice with 20-ml. portions of Skelly

solve B. The extracts are combined and washed three times with small quantities of distilled water. This washed ether extract is concentrated under partial vacuum to less than 25 ml., after which it is dried over anhydrous sodium sulphate. The extract is then transferred to a glass-stoppered graduated cylinder. Hexane is added to make the volume up to approximately 25 ml., the dilution depending on the intensity of the color needed for comparison.

The carotene content of this solution is determined by means of a spectrophotelometer.

Since this procedure was developed, other investigators (13, 17) have employed the Roese-Gottlieb principal of extraction in determining the carotene of milk and milk products.

Calculations. The quantity of carotene in the pasture herbage was calculated as micrograms per gram of dry matter and that for milk, standardized at four-per-cent fat, as micrograms per liter. Since this four-per-cent standard is frequently used in dairy calculations, it was adopted as the common base for expressing the carotene content of the milk produced by the three different breeds.

For each grazing system, the carotene data for the milk from the three breeds and for the pasture herbage were grouped chronologically by semimonthly periods for each grazing season. Subsequently these data for the three seasons were averaged by the corresponding calendar periods. This organization of the data was designed to show trends ascribable to seasonal changes as well as to indicate variations resulting from the different systems of grazing.

RESULTS

The results as shown in the graphical summary of the data, figure 1, are essentially negative; that is, the system of grazing effected no marked and consistent differences in the carotene of either the pasture plants or the milk.

In the case of the carotene concentration of the herbage, the only notable difference that could be related to the system of grazing occurred in June when there was a transition from leguminous plants to Bermuda grass. Since the carotene of plants generally bears a direct relationship to their rate of growth, the observed difference at this period might be ascribed to the fact that the continual grazing retarded the rate of recovery of the pasture herbage to a greater degree than did the rotational grazing.

Irrespective of the system of grazing, the trends and fluctuations in the carotene of the herbage reflected the influences of several interrelated modifying factors, most pronounced of which were stage of maturity and rainfall. When the clovers and vetches were flourishing in the spring and Bermuda grass was in its initial stages of growth in the early summer, the carotene concentrations were higher than at any other time. The effect of maturation, accompanied by droughts in late spring and early fall, was indi-

cated by the interrupted decreases in the Bermuda grass, first, following the initial flush in June and, second, after the marked growth retardation in early fall.

A comparison of the carotene values of the milk produced from the respective systems of grazing disclosed no pronounced differences that might be ascribed to the systems of grazing. This observation is in accord with expectations since the yield (18) and the carotene values of the herbage from the comparative systems were similar.

The trends of the carotene of the milk, for the most part, were typical for normal pasture grazing. The precipitous rise during the first two weeks following the change of the feeding regime from winter roughage to pasture herbage is in agreement with long-established precedent (14), but the subse-

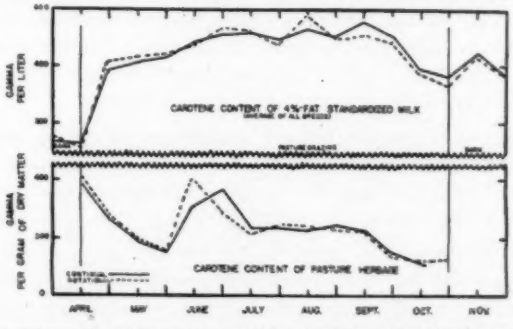

FIG. 1. Summary of a three-year pasture investigation showing the comparative effects of continual and of rotational grazing on the carotene concentration of the pasture herbage and of the milk produced therefrom.

quent retarded rate of increase to the maximum plateau, apparently the "ceiling" value (16), is somewhat unusual (11). Evidently a decrease in the carotene intake resulting from the maturation of the spring legumes was the primary factor contributing to this retardation. A probable secondary operative element was the increased output in the milk; even though the amount of carotene eliminated through this channel constitutes only a small fraction of the total amount ingested (3, 4, 6, 8, 12, 15). The changes in the milk yield during May and June were incommensurate with the reduction in the availability of fresh green herbage. Therefore, the additive results of these relative changes in intake and output would account, in a great measure, for the prolongation of the time necessary to reach equilibrium.

The temporary interruption of the decline of carotene concentration of the milk coinciding with the change from grass to silage might be attributed primarily to an increased intake of carotene. The fresh silage was more palatable and probably more potent in carotene than the Bermuda grass in an advanced stage of maturity.

DISCUSSION

The emphasis placed on the rôle of carotene as the primary source of vitamin A active substances in dairy cattle nutrition has led to the exploration of a multitude of factors affecting the concentration of this compound in the plants commonly used for dairy feeds. Grazing *per se* probably affects the carotene of permanent pasture herbage to the extent that it modifies the direct influence of other factors. Grazing practices that either impair plant development or permit the plants to reach an advanced stage of maturity obviously adversely affect carotene values.

In the foregoing comparison of continual and rotational systems of management the intensity of grazing was regulated so that optimum returns were obtained from lactating cows throughout the grazing season. Since the carrying capacity of the pastures under the two systems of grazing was approximately the same, the similarity in carotene values was in accord with expectations when these observations are considered in the light of the vital rôle that the quality of forage plays in milk production.

It is reasonable to assume that any pasture providing the quality and the quantity of herbage necessary to maintain optimum milk yields over a prolonged period also would supply carotene in sufficient amounts to raise its concentration in the milk to the "ceiling" value. Irrespective of system of grazing the uniform level of carotene in the milk (figure 1) during the summer months and the lag in its reduction in relation to the initial decrease of carotene in the herbage, during the latter part of summer, tend to support the foregoing assumption. However, a possibility that cannot be disregarded entirely is that the intake-output relationship of the carotene during the summer months might have been so constant that a uniform level in the carotene of the milk was maintained at a "sub-ceiling" level.

During the latter part of the grazing season, the decrease in the carotene of the milk following that of the herbage indicates that either the cows did not have a carotene reserve or that the reserve was not being utilized in sufficient amounts to maintain the prevailing high level in the milk. This last suggestion appears to be the more rational explanation in view of the present knowledge of depletion of body stores.

Even though the observations of this investigation disclosed no significant differences that might be ascribed to the system of grazing, the results do not preclude the possibility that the two systems may reveal a different relationship, particularly in the carotene of the herbage, with other species of plants and/or different soils and fertilizers.

SUMMARY

A study was made of the relation of continual and rotational systems of grazing to the carotene content of the herbage of improved Bermuda grass

pastures and of the milk produced by respective groups of cows grazing thereon.

The system of grazing effected no marked and consistent difference in either the concentrations or the variations of the carotene in the herbage.

The carotene concentrations and fluctuations of the milk revealed no differences that could be ascribed to the system of grazing.

The quantitative changes in the carotene ingested were reflected in the carotene values of the milk during the spring and the fall when the concentrations in the milk were at a "sub-ceiling" level, but this did not hold true during the summer, since the values of the milk apparently were at "ceiling" level.

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OXIDIZED FLAVOR IN MILK. XIII. STUDIES OF CUPRIC COMPLEXES OF ASCORBIC ACID AND CERTAIN AMINO ACIDS AND THEIR POSSIBLE RELATIONSHIP TO OXIDIZED FLAVOR DEVELOPMENT IN MILK*

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In studies on the mechanism for the production of oxidized flavor in milk Olson and Brown (4) postulated that the ascorbic acid in milk was oxidized by copper ions giving hydrogen peroxide as a product; the hydrogen peroxide was then thought to oxidize the phospholipides of milk, producing the oxidized flavor. It was found, however, that excess ascorbic acid prevented the formation of oxidized flavor. This would seem to be an anomaly. However, this might be explained if ascorbic acid formed a complex ion with copper.

Wark (7), in studying the complex ions of hydroxy acids with copper, stated: "The capability of any hydroxy-compound to form complexes with metallic oxides must depend on the acidity of the hydroxyl group. The more strongly this group functions as an acid, the more stable will the complexes be." From this it would seem that the highly acidic hydroxy group of ascorbic acid could form a complex.

Morton (3) reported that in neutralized solutions copper complexes were formed with tartaric, citric, malic, and salicylic acids. In alkaline solution these complexes were destroyed with the formation of highly basic hydrosols. In contradiction to Wark (7) Morton further stated that no stable copper complexes were formed with glyceric, lactic, glycollic, or mandelic acids, the apparent stability of the cupric salts of these hydroxy acids in the presence of caustic alkalies being due to the peptizing properties of the hydroxy-acid anion.

Any compound that forms a copper complex would help prevent the oxidation of the normal ascorbic acid in milk and thus inhibit the formation of oxidized flavor. Sherwood and Hammer (5) have reported concentrations of citric acid salts ranging from 0.07 per cent to 0.33 per cent in milk. In addition, the proteins of milk should form copper complexes although there is some dispute as to the type of complex formed.

Proteins may form complexes with copper through the free amino groups. Thus they would act similarly to amino acids. Borsook and Thimann (1)

Received for publication August 5, 1943.

* Published with the approval of the Director of the West Virginia Agricultural Experiment Station as Scientific Paper No. 309.

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studied the equilibrium relations existing in solution between copper ions and glycine and alanine. They were studied by measurement of absorption spectrum and by copper electrode potentials through a range of hydrogen ion concentrations from pH 0 to 13. They found four types of complexes of both glycine and alanine, depending on the pH of solution.

Smythe and Schmidt (6) studied the formation of complexes of sixty-one compounds with iron. This would be similar to the complexes of copper. By calculating the residual negative charge of the nitrogen atom in amino acids, they reasoned that monoamino monocarboxylic acids would not form a complex. Their experimental work confirmed this. However, they found that dicarboxylic acids and hydroxy acids did form complexes. In addition they claimed the formation of a complex with phosphoric acid and iron due to the residual negative charges on the hydroxy groups of phosphoric acid. The formation of an iron complex with casein was claimed to be due to the phosphoric acid in casein. These studies were done at pH 2.5.

The purpose of the present study was to determine whether or not copper complexes were formed with ascorbic acid and amino acids. Citric acid was also used for comparative purposes. β-alanine was used to compare the action of α-amino and β-amino acids.

EXPERIMENTAL

Most determinations of copper complexes are done with relatively high concentrations of copper. In this work it was desired to work in the range of copper concentration that might be found in milk, that is, about one part per million. For this purpose a method for determining very small amounts of ionized copper was sought. A modified Biazzo (2) method was found applicable. By using a 10-centimeter tube in a Keuffel and Esser color analyzer at a wave length of 450 mμ., a range of copper concentration from 0.01 mg. to 0.1 mg. was accurately determined. The copper content of the water and reagents was estimated by using a water blank with the color reagents against the different copper concentrations. By plotting $-\log T$ against concentration of copper a straight line parallel to the line obtained by running the various copper concentrations against a chloroform blank was obtained. From the distance between the lines the copper content of the reagents and water was found to be from 0.01 mg. to 0.015 mg. for the various batches of redistilled water used.

Although it was desirable to determine the amount of copper ionized at pH 6.5 to 7.0, this method (2) used acetic acid for the development of color and, consequently, the amount of copper ionized was actually determined at a pH of about 3.5. According to Morton (3) the complex of copper should be determined at neutrality. Ascorbic acid and citric acid with copper showed a diminution of copper ions, but these values would have to be considered minimum values. The amino acids showed very little diminution

of copper ions at the pH of reaction. Since Borsook and Thimann (1) have shown that a different complex of amino acids is formed at pH 3.5 than at pH 6.5, this method was not used for the determination of the amino acid complexes.

The amount of ionizable copper with citric acid was determined by adding concentrations of 0.1 per cent to 1.0 per cent of citric acid neutralized to pH 6.5, to 0.1 mg. of copper and adding the reagents to develop color to a total volume of 100 ml. The color was extracted from the water solution with 25 ml. of chloroform, the chloroform was centrifuged for a few min-

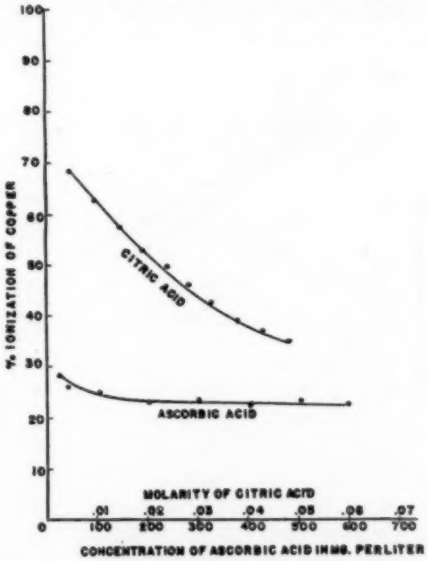

FIG. 1. Ionization of copper with citric and ascorbic acids.

utes to separate water droplets, and the color was immediately read in the color analyzer.

In a similar way the ionization of copper was determined with ascorbic acid, using concentrations of 20 mg. to 600 mg. per liter.

The results of both the citric acid and the ascorbic acid are shown in figure 1. They are calculated as the percentage of the total copper ionized. The curve for citric acid shows results typical for a complex, that is, the higher the concentration of citric acid the less copper was ionized. The ascorbic acid shows an entirely different type of curve. The amount of ionizable copper remains practically the same for all the concentrations of ascorbic acid. Instead of a typical complex like that of citric acid, the ascorbic acid seems to form a definite compound of copper ascorbate. The ascorbic acid ties up all but about 25 per cent of the copper very definitely.

It was desired to see what effect the copper complex of citric acid had on the protection of ascorbic acid. In a preliminary trial it was found that copper in a concentration of one part per million in 13 minutes completely destroyed, 40 mg. per liter of ascorbic acid in a 0.05 m. phosphate buffer of pH 6.8. This 13-minute period was therefore used as a comparative time interval. A series of 100-ml. volumetric flasks containing 0.1 mg. copper, the phosphate buffer at pH 6.8 and neutralized citric acid in concentrations of 0.1 per cent to 1.0 per cent was set up. Ascorbic acid to make a concentration of 40 mg. per liter was added, the flask filled to volume quickly,

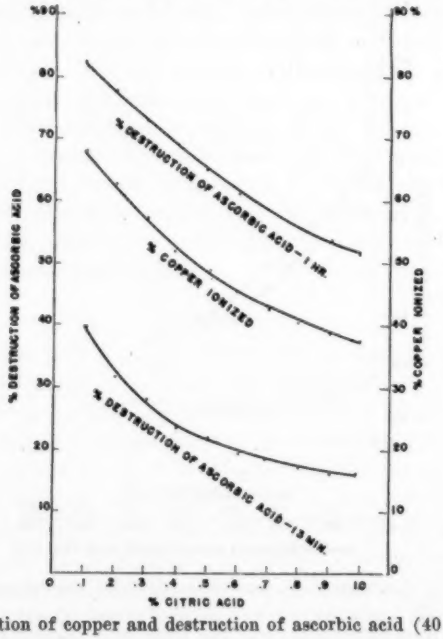
% DESTRUCTION OF ASCORBIC ACID
% DESTRUCTION OF ASCORBIC ACID – 1 HR.
% COPPER IONIZED
% DESTRUCTION OF ASCORBIC ACID – 13 MIN.
% COPPER IONIZED
% CITRIC ACID

FIG. 2. Ionization of copper and destruction of ascorbic acid (40 mg./l.) with citric acid.

mixed, and placed in the dark. After exactly 13 minutes an aliquot was removed and titrated with a solution of sodium 2.6-dichlorobenzenoneindophenol to determine the amount of ascorbic acid remaining. A similar aliquot was titrated after one hour.

The results are shown in figure 2. For comparison the curve for ionizable copper with citric acid is also shown. It is readily seen that less ascorbic acid is destroyed with increasing concentration of citric acid. The citric acid protects the ascorbic acid from destruction. In addition the two curves for the destruction of ascorbic acid are similar in shape to the curve for ionizable copper, particularly the curve for one hour. These curves

show definitely that the inhibition of ascorbic acid destruction is due to the removal of copper ions by the citric acid complex.

The same method was used to determine whether or not amino acids prevent the destruction of ascorbic acid by copper. The experiments were conducted exactly the same as for citric acid, using glycine, glutamic acid, alanine, and β-alanine in the same molar concentrations. Thus results were obtained for two monoamino monocarboxylic amino acids, one monoamino dicarboxylic acid, and one β-amino acid. Citric acid was also run for comparative purposes. The results are the average of two or three determinations.

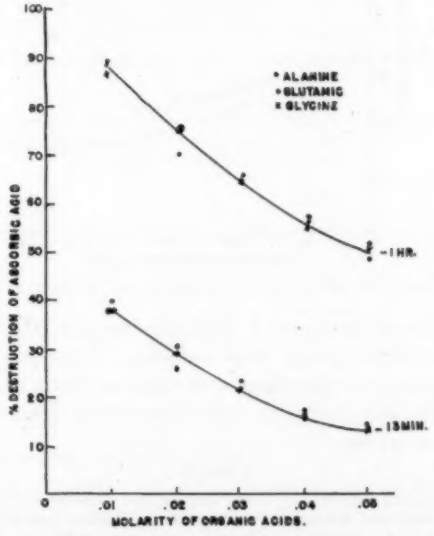

FIG. 3. Protection of ascorbic acid by complex ions of α-amino acids.

The results of the amino acids as inhibitors of the destruction of ascorbic acid are shown in figures 3 and 4. It will be noted in figure 3 that glycine, glutamic acid, and alanine all fall along the same curves. The variation from the curves is no more than the experimental error in the individual determinations. It may be noted in figure 4 that citric acid forms a curve of different shape from the amino acids (glycine, glutamic, and alanine) but of close to the same protective value. Glutamic acid gives the same curve as glycine and alanine, contrary to Smythe and Schmidt's (6) hypothesis that the two carboxyl groups form the complex and not the amino group. Furthermore it must be remembered that the amino acids are compared to the destruction in phosphate buffer which completely destroys the ascorbic acid in thirteen minutes. Smythe and Schmidt's (6) contention that phosphoric acid forms a copper complex is not supported by these results.

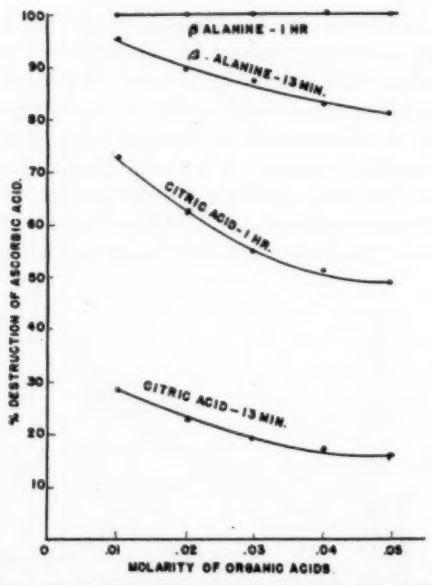

FIG. 4. Protection of ascorbic acid by complex ions of citric acid and β-alanine.

Finally the curves in figure 4 for β-alanine show that the amino group in the β position forms a very weak complex. After one hour the ascorbic acid is totally destroyed regardless of the concentration of β-alanine. These results would definitely indicate that amino acids form copper complexes through the amino group.

TABLE 1

The effect of varying amounts of citric acid on the development of oxidized flavor in buffered washed cream containing 42 mg. per liter of ascorbic acid and 1.0 ppm. copper

	Date					
	8/19/42	8/20/42	8/21/42	1/9/43	1/12/43	1/15/43
Control	ox	ox	ox	ox	ox	ox
Washed cream plus citric acid						
0.05%	.....	.....	ox	.....	.....	.....
0.1%	ox	ox	ox	.....	.....	.....
0.2%	?	sl. ox	sl. ox	ox	ox	sl. ox
0.3%	.....	.....	?	.....	.....	.....
0.4%	.....	.....	–	.....	.....	.....
0.5%	–*	?	–	–*	sl. ox	–
0.6%	.....	.....	.....	.....	.....	.....
0.7%	–*	–*	.....	.....	.....	.....
0.8%	.....	.....	.....	.....	.....	.....
0.9%	–*	–*	.....	.....	.....	.....
1.0%	–*	–*	.....	–*	–	.....

Meaning of symbols: * bitter from citrate; ox oxidized flavor; – no oxidized flavor.

TABLE 2

The effect of citric acid on the development of oxidized flavor in susceptible milk contaminated with 1.0 ppm. copper

	Date				
	1/8/43	1/14/43	1/18/43	1/19/43	1/20/43
Control	ox*	ox	ox	ox	ox
Milk plus citric acid 0.02 M.	–	–	–	–	–

* For meaning of symbols, see table 1, footnote.

Since citric acid has been shown to have a protective action against the oxidation of ascorbic acid by the copper ion it seemed desirable to determine its effect on the development of oxidized flavor in washed cream and milk. Accordingly, samples of washed cream were prepared and tested according to the method of Olson and Brown (4). The results of these experiments

TABLE 3

The effect of adding alanine and glycine on the susceptibility of washed cream containing 42 mg. per liter of ascorbic acid and 1.0 ppm. copper to the development of oxidized flavor

	Date				
	12/31/42	1/9/43	1/12/43	1/15/43	1/19/43
Control	ox*	ox	ox	ox	ox
Conc. of alanine					
0.02 M.	ox	ox	sl. ox	sl. ox	ox
0.05 M.	sl. ox	sl. ox	sl. ox	–	sl. ox
0.10 M.	.....	?	–	–	v. sl. ox
Conc. of glycine					
0.02 M.	ox	ox	ox	sl. ox	sl. ox
0.05 M.	sl. ox	sl. ox	sl. ox	sl. ox	?
0.10 M.	.....	?	v. sl. ox	–	–

* For meaning of symbols, see table 1, footnote.

are shown in tables 1 and 2. These results confirm the finding that citric acid has an inhibitory effect on the development of oxidized flavor by ascorbic acid.

Likewise, the effect of alanine and glycine was studied on both washed cream and milk. These results are shown in tables 3 and 4.

TABLE 4

The effect of adding alanine and glycine on the susceptibility of milk containing 1.0 ppm. of added copper to the development of oxidized flavor

	Date				
	1/8/43	1/14/43	1/18/43	1/19/43	1/20/43
Control	ox*	ox	ox	ox	ox
Conc. of alanine					
0.02 M.	–	.....	.....	.....	.....
0.05 M.	–	.....	.....	.....	.....
Conc. of glycine					
0.02 M.	.....	–	–	–	–
0.05 M.	.....	–	–	–	–

* For meaning of symbols, see table 1, footnote.

Here, likewise, both alanine and glycine are found to have an inhibitory action on the development of oxidized flavor. This inhibitory action was not as strong as in the case of the citric acid.

DISCUSSION OF RESULTS

These results indicate that the development of oxidized flavor in milk is closely associated with the ionization of copper and its destruction of ascorbic acid. Apparently anything which decreases the ionization of copper will in turn retard the destruction of ascorbic acid and in this manner tend to retard oxidized flavor development. As a result of these findings, it seems that many individual factors may have a bearing on the susceptibility or nonsusceptibility of milk to the development of oxidized flavor.

CONCLUSIONS

1. Ascorbic acid forms either a complex or a direct compound with copper ions.

2. The protective action of citric acid on the oxidizing action of copper ions with ascorbic acid is due to the formation of a copper complex with citric acid thus removing copper ions from the reaction.

3. Through the indirect evidence of inhibition of ascorbic acid destruction with copper ions, it is shown that the amino acids—glycine, glutamic acid, and alanine—are equally effective in forming complex ions. With the evidence that β-alanine forms a very weak complex, this shows that the amino acids form copper complexes through the free amino group.

ACKNOWLEDGMENT

The authors are indebted to Dr. R. B. Dustman, Department of Agricultural Chemistry, for his assistance and constructive criticism in the preparation of this paper.

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OXIDIZED FLAVOR IN MILK. XIV. A POSSIBLE MODE OF ACTION OF INHIBITORS IN PREVENTING THE DEVELOPMENT OF OXIDIZED FLAVOR IN MILK*

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Received for publication August 5, 1943.

Various substances have been found to inhibit the formation of oxidized flavor in milk. Dahle and Palmer (5) found that excess ascorbic acid protects against oxidized flavor. Anderson (1) reported that a pancreatic enzyme added to milk just before pasteurization protects the milk against oxidized flavor. Corbett and Tracy (4) found that both tyrosine and the butyl ester of tyrosine prevented the flavor, and that "Enzylac" and Avenex gave protection. Even ordinary raw sugar gave some protection. Williams and Burgwald (16) found that carotene did not protect, but that mixed tocopherols gave a protection. Garrett (7) found that divalent manganese protected milk against oxidized flavor. Archibald (2) fed an ounce of manganese sulphate daily to cows and analyzed the milk. The manganese content of the milk was doubled, but even this amount was not sufficient manganese to prevent oxidized flavor. Brown and Olson (3) found that potassium iodide added directly to the milk protected against oxidized flavor.

During the summer of 1942 the authors attempted to isolate the active inhibitor in summer milk. The casein was precipitated at the isoelectric point. This was made into calcium caseinate by the method of Palmer (13). The calcium caseinate, whey, and sterilized whey, were then tested on washed cream according to the method of Olson and Brown (12). In most cases the oxidized flavor was prevented. In addition lactose was tried and found to inhibit oxidized flavor. Thus it was found that a number of naturally occurring substances in milk inhibit oxidized flavor.

Brown and Olson (3) proposed a chemical theory for the development of oxidized flavor in milk. This theory postulated that cupric copper oxidizes the ascorbic acid in milk to dehydroascorbic acid with the production of copper in the cuprous state of ionization. The cuprous copper is then oxidized back to the cupric form by dissolved oxygen with the production of hydrogen peroxide. The hydrogen peroxide in turn oxidizes the phospholipides in milk to give the oxidized flavor.

The purpose of this paper was to study the natural inhibitors in milk as well as the inhibitors proposed by other workers in order to test this theory

* Published with the approval of the Director of the West Virginia Agricultural Experiment Station as Scientific Paper No. 310.

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of oxidized flavor development. To test this the inhibitors were tried again on milk and wherever possible on washed cream to be sure that a preventive for oxidized flavor was actually present. A chemical explanation was then attempted for the inhibiting action.

Referring again to the theory of the development of oxidized flavor, it is readily seen that it can be prevented (I) by removal of copper ions so that the ascorbic acid is not oxidized. A class of inhibitors in this group are those that form complex ions with copper thus reducing the copper ions in solution. A second class (II) would include substances that are themselves oxidized by hydrogen peroxide before the hydrogen peroxide oxidizes the phospholipides of milk. Because of a lack of knowledge of the chemistry involved, a third class (III) must be added to include substances that inhibit oxidized flavor but for which the mechanism is not understood.

EXPERIMENTAL RESULTS

I. The first class of inhibiting substances in which the ionization of copper is repressed is typical of salts of citric acid and amino acids as cited by Olson and Brown (11). They found that hydroxy organic acids form a complex with copper which inhibits the ionization. This in turn inhibits the oxidation of ascorbic acid and consequently the oxidized flavor formation. The effect of amino acids was tried in order to gain an insight into the action of proteins. From the results with amino acids it is seen that the inhibiting effect of proteins would be caused by the free amino and carboxyl groups of the proteins.

Casein. Calcium caseinate, made according to the method of Palmer (13), was tried as an inhibitor on washed cream according to the method of Olson and Brown (12). The results are shown in table 1. These results show that calcium caseinate in a three per cent solution protects washed cream against oxidized flavor. In two cases oxidized flavor was inhibited by a percentage lower than the casein content of milk. This would indicate that in the preparation of the calcium caseinate, it was partly hydrolyzed and the liberated free amino groups inhibited the oxidized flavor.

Proteolytic enzymes. Since "Enzylac" includes a proteolytic enzyme, the inhibiting effect of this product may be due to a partial hydrolysis of milk proteins which liberate free amino groups to form complex ions with copper. That "Enzylac" does protect milk against oxidized flavor is shown in table 2 where the enzyme was added according to the directions of the manufacturer.

Ascorbic acid. Another substance belonging to this class is ascorbic acid, as shown by the results of Olson and Brown (11). The presence of excess ascorbic acid prevents the ionization of copper probably through the formation of a copper compound. In this manner the ascorbic acid differs from the amino acids which form complexes as indicated by decreased ionization of copper with increased concentration of the amino acids.

TABLE 1

The effect of various concentrations of calcium caseinate on the susceptibility of washed cream containing 42 mg./liter of ascorbic acid to oxidized flavor

Date	Control Washed cream ppm. Cu				3% calcium caseinate plus ppm. Cu				2% calcium caseinate plus 1.0 ppm. Cu	1% calcium caseinate plus 1.0 ppm. Cu
	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5		
7/30/42	ox*	ox	ox	ox	–	–	–	–	.....	.....
8/12/42	sl. ox	ox	ox	ox	–	–	sl. ox	ox	.....	.....
8/14/42	ox	ox	ox	ox	–	–	–	–	.....	.....
12/30/42	ox	ox	ox	ox	.....	.....	sl. ox	.....	sl. ox	sl. ox
1/15/43	ox	ox	ox	ox	.....	.....	–	.....	–	–
1/19/43	ox	ox	ox	ox	.....	.....	–	.....	–	–

* Meaning of symbols: ox, oxidized flavor; –, no oxidized flavor.

II. The second class of substances includes those which inhibit oxidized flavor by the substances themselves being oxidized by hydrogen peroxide such that the phospholipides of milk are not oxidized. Lactose, avenex, potassium iodide, and manganese showed very little or no protection of ascorbic acid against oxidation when tried according to the method used by Olson and Brown (11) and therefore do not form complexes with copper. Tyrosine showed a very slight effect in 0.5 per cent suspension but no more than could be attributed to the small amount of amino acid present in solution.

Tyrosine. The effect of tyrosine as an inhibitor of oxidized flavor both with washed cream and milk is shown in table 3. The results show that 0.1 gram of tyrosine per half pint inhibits oxidized flavor in both milk and washed cream although it may not entirely eliminate the flavor. Since tyrosine is quite insoluble, no higher concentration could be tried. The action of tyrosine as an inhibitor is explained by Mystkowski (10). He has shown that tyrosine added to a solution of ascorbic acid and copper inhibits oxygen evolution and the tyrosine changes to a reddish brown color corresponding to quinone products of tyrosine oxidation. Thus it is seen that tyrosine inhibits oxidized flavor in milk by removing the hydrogen peroxide

TABLE 2

The effect of "Enzylac" as an inhibitor of oxidized flavor on susceptible milk

Date	Control milk				Milk + "Enzylac"			
	ppm. Cu				ppm. Cu			
	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5
1/11/43	–	ox	ox	ox	–	–	–	–
1/14/43	–	ox	ox	ox	–	–	–	–
1/19/43	–	ox	ox	ox	–	–	–	–
1/20/43	–	–	ox	ox	–	–	–	–

For meaning of symbols, see table 1, footnote.

formed in the ascorbic acid oxidation. Avenex, being a natural product mixture, might contain some quinone type compound which could prevent oxidized flavor similar to tyrosine.

Manganese. It would seem to be very strange that divalent manganese would be an inhibitor of oxidized flavor. However, the results in table 4 show that manganese in sufficient quantities definitely prevents oxidized flavor in milk. The following explanation seems possible. When sodium hydroxide is added to a divalent manganese salt a light tan precipitate immediately forms. On exposure to air for a few minutes the top of the precipitate turns black with the formation of $MnO(OH)_2$ due to oxidation by atmospheric oxygen. Similarly if a manganese salt is added to a citrate buffer at a pH of 6.5–7.0, a very light-colored solution results. On standing

TABLE 3

The effect of the addition of 0.1 gram of tyrosine per half-pint on the development of oxidized flavor in milk and washed cream

Date	Milk								Washed cream (buffered)		Tyrosine
	Control				Milk + 0.1 gr. tyrosine				Control	Washed cream+tyrosine	
	ppm. Cu				ppm. Cu				1.0 ppm. Cu	Plus 42 mg./l. ascorbic acid 1.0 ppm. Cu	
	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5			
12/31/42	–*	–	–	–	.....	.....	.....	.....	ox	ox	0.01 M
1/ 8/43	–	ox	ox	ox	–	–	–	–	.....	.....	.....
1/12/43	.....	.....	.....	.....	.....	.....	.....	.....	ox	ox	0.1 gr.
1/14/43	–	ox	ox	ox	–	–	–	sl. ox	.....	.....	.....
1/15/43	.....	.....	.....	.....	.....	.....	.....	.....	ox	–	0.1 gr.
1/18/43	–	–	ox	ox	–	–	–	–	.....	.....	.....
1/19/43	–	–	ox	ox	–	–	–	–	.....	.....	.....
1/20/43	–	–	ox	ox	–	–	–	–	.....	.....	.....

* For meaning of symbols, see table 1, footnote.

in the air this gradually becomes a dark brown, indicating oxidation by atmospheric oxygen. Thus manganese either protects milk from oxidized flavor by absorbing the hydrogen peroxide in the ascorbic acid oxidation process, or it may even absorb the dissolved oxygen in the milk thus preventing the oxidation of the copper to form hydrogen peroxide.

Sugars. Lactose shows some inhibition of oxidized flavor as is seen in table 5. Since lactose does not form a complex ion with copper, the only possible explanation for this effect is that the potentially free aldehyde group in lactose is oxidized by hydrogen peroxide. To test this further, sucrose was tried on washed cream. Sucrose does not have a potential aldehyde group and it did not inhibit oxidized flavor. Glucose on the other hand is a simple sugar with a potential aldehyde group. As shown in table 6, glucose did tend to prevent oxidized flavor. That glucose is capable of being oxidized by hydrogen peroxide was shown by Witzemann (17) who found that glucose could be completely oxidized to carbon dioxide and water by

TABLE 4

The effect of various concentrations of manganese on the susceptibility to oxidized flavor of milk and washed cream contaminated with copper

Date	Milk									
	Control + Cu				Control + 1.5 ppm. Cu + Mn					
	ppm. Cu				ppm. Mn					
	0.0	0.5	1.0	1.5	0	1	2	3	4	5
1/ 8/43	–*	ox	ox	ox	ox	–	–	–	.....	.....
1/14/43	–	ox	ox	ox	ox	ox	–	–	.....	.....
1/18/43	–	sl. ox	sl. ox	ox	ox	–	–	–	.....	.....
1/19/43	–	–	ox	ox	ox	ox	–	–	.....	.....
1/20/43	–	–	ox	ox	ox	–	–	–	.....	.....
	Washed cream (buffered) containing added ascorbic acid, 42 mg./l.									
					Control + 1.0 ppm. Cu + Mn					
					ppm. Mn					
					0	1	2	3	4	5
1/ 8/43	.....	.....	.....	.....	ox	ox	ox	sl. ox	.....	.....
1/12/43	.....	.....	.....	.....	ox	ox	ox	sl. ox	v. sl. ox	.....
1/15/43	.....	.....	.....	.....	ox	ox	sl. ox	–	–	–

* For meaning of symbols, see table 1, footnote.

hydrogen peroxide in the presence of disodium phosphate in a pH range of 5.9 to 7.35. If this is the case, it would seem to be easy for the small amount

TABLE 5

The effect of added lactose on the susceptibility of oxidized flavor in milk and washed cream

Date	Control milk				Milk + 2½% lactose				ppm. Cu			
	ppm. Cu				ppm. Cu				Milk + 5% lactose			
	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5
1/ 8/43	–*	ox	ox	ox	–	–	–	–	.....	.....	.....	.....
1/14/43	–	ox	ox	ox	.....	.....	.....	.....	–	–	–	–
1/18/43	–	–	ox	ox	.....	.....	.....	.....	–	–	–	–
1/19/43	–	–	ox	ox	–	–	sl. ox	sl. ox	.....	.....	.....	.....
1/20/43	–	–	ox	ox	.....	.....	.....	.....	–	–	–	sl. ox
	Washed cream + 42 mg./l. ascorbic acid				Washed cream + 32 mg./l. ascorbic acid + 2½% lactose				Washed cream + 42 mg./l. ascorbic acid + 5% lactose			
8/20/42	ox	ox	ox	ox	–	–	–	–	–	–	–	–
8/21/42	ox	ox	ox	ox	–	–	–	–	–	–	–	–
1/ 9/43	ox	ox	ox	ox	.....	.....	ox	.....	.....	.....	ox	.....
1/12/43	ox	ox	ox	ox	.....	.....	sl. ox	.....	.....	.....	sl. ox	.....
1/15/43	ox	ox	ox	ox	.....	.....	ox	.....	.....	.....	–	.....
1/19/43	ox	ox	ox	ox	.....	.....	sl. ox	.....	.....	.....	v. sl. ox	.....

* For meaning of symbols, see table 1, footnote.

of hydrogen peroxide formed in the oxidation of ascorbic acid in milk to be used to oxidize glucose or lactose and thus protect from oxidized flavor. That the lactose in milk does not entirely prevent oxidized flavor would be due to both oxidation processes going on at the same time.

The protection from oxidized flavor by tocopherols as found by Williams and Burgwald (16) would be included in this second class of inhibitors also. The tocopherols have a hydroquinone type of structure and are very easily oxidized. The problem as an antioxidant for milk would be to get the tocopherols, which are oil soluble, into a sufficiently fine emulsion to give enough surface for antioxidant properties.

TABLE 6

The effect of glucose on the susceptibility of milk and washed cream to oxidized flavor

Date	Control milk	Milk + 0.5% Glucose	Milk + 1.0% Glucose	Milk + 2.0% Glucose
	ppm. Cu	ppm. Cu	ppm. Cu	ppm. Cu
	0.0 0.5 1.0 1.5	0.0 0.5 1.0 1.5	0.0 0.5 1.0 1.5	0.0 0.5 1.0 1.5
1/18/43	–* – ox ox	– – – –	– – – –	– – – –
1/19/43	– – ox ox	– – – ox	– – – ox	– – – †
1/20/43	– – ox ox	– – – †	– – – –	– – – –
	Washed cream + 42 mg./l. asc. acid No glucose	Washed cream + 42 mg./l. asc. acid 2½% glucose	Washed cream + 42 mg./l. asc. acid 5% glucose	
	ppm. Cu	1.0 ppm. Cu	1.0 ppm. Cu	
	0.0 0.5 1.0 1.5			
1/19/43	ox ox ox ox	sl. ox	sl. ox	

* For meaning of symbols, see table 1, footnote .

In connection with this second class of inhibitors, there is also a possibility that an inhibitor may directly oxidize ascorbic acid and thus eliminate the oxidation step by copper. In this way hydrogen peroxide would not be formed and no oxidized flavor in milk would develop. Heard and Welch (8) have found such a system in effect in the adrenal glands. They found that the ascorbic acid directly reduced oxidized adrenaline, forming reduced adrenaline and dehydro-ascorbic acid.

III. A miscellaneous class of inhibitors, which includes those that do not fit well into either group one or group two, should also be considered. In this group are the halides. Brown and Olson (3) found that potassium iodide inhibited oxidized flavor to some extent. Mapson (9) found that the halides—iodides, bromides, and chlorides—showed a protective action on ascorbic acid at pH 2.5 but very little at pH 6 to 7. Mystkowski (10) found good inhibition by sodium chloride in concentrations of 4 per cent to 10 per

cent even at pH 7. This may explain why salted cream used for butter does not develop oxidized flavor. The mechanism of this protective action of the halides is not known. Mapson (9) has postulated a complex ion of the halide with copper. However, the authors were unable to show any diminishing of the ionization of copper in a solution of potassium iodide, using the method of Olson and Brown (11).

Substances that prevent oxidized flavor by themselves being oxidized by the hydrogen peroxide are of interest. These substances in order to be effective must have an oxidation-reduction potential such that they will be oxidized before the phospholipides. Tyrosine has such an action, but (tyrosine) is not sufficiently soluble in milk to completely prevent the development of oxidized flavor. Divalent manganese, as was found by Garrett (7), inhibited oxidized flavor. An explanation of this is the oxidation of manganous ions to manganic ions by the hydrogen peroxide. That lactose could inhibit oxidized flavor would seem unlikely but the oxidation of the aldehyde group of the sugar and even further oxidation has been shown by Witzemann (17) with glucose.

Since the proteins, citrates, and lactose in milk all have an inhibiting effect on oxidized flavor, it is natural to expect that an increase in any or all of these substances would show an inhibiting effect on winter milk. The work of Russell and Dahle (15) in which added milk solids in the form of condensed milk or dried milk inhibited oxidized flavor development, may be explained on this basis. However, since the condensed milk and dried skimmilk may have been subjected to a sufficiently high temperature to liberate sulfhydryl groups, these also might explain the inhibiting action.

DISCUSSION

The results show that a number of substances normally occurring in milk act as inhibitors. These substances—proteins, citrates, and lactose—are present in about the same amount in both summer and winter milk. They show a definite inhibiting action on washed cream. The proteins, particularly, as well as the citrates, to a smaller extent, inhibit by forming complexes with copper, thus removing the copper ions from the reaction. These complexes are formed by the free amino and carboxyl groups of the proteins similar to amino acids. Peterson and Walton (14) have found that various amino acids as well as uric acid inhibit the oxidation of ascorbic acid with copper by forming ions with copper. That it was a complex of copper was shown by the fact that the normal autoxidation of ascorbic acid was not inhibited by the amino acids in the absence of copper. They found that 10^{-4} M cystine was the best inhibitor. Diehl (6) has included a theoretical discussion of copper complexes of amino acids and hydroxy acids through the coordinating groups so as to form chelate rings. These complexes prevented the ionization of copper.

Sinee proteins inhibit oxidized flavor by the formation of complexes with copper through the free amino and carboxyl groups, any proteolytic enzyme that splits the protein to give more free reactive groups would show a further inhibiting action. This is true of "Enzylac." That the inhibiting effect is not due to the preparation itself is shown by the fact that it does not inhibit oxidized flavor in washed cream.

The search for a special inhibitor present in milk from cows on pasture is complicated by the fact that the normal constituents of milk have an inhibitory action. Such a special inhibitor is entirely possible. However, the results showing that citrates, proteins, and lactose have an inhibitory effect on milk might indicate that the tendency to form oxidized flavor is balanced by these natural inhibitors. Although Brown and Olson (3) found that washed cream containing ascorbic acid and contaminated with copper gives oxidized flavor on pasture milk, the amount of oxidized flavor seemed to be less than that produced on winter milk. This suggests that the phospholipides in winter milk may be more unsaturated than in summer milk. One of the authors has started work on this consideration.

SUMMARY

A possible explanation for various inhibitors for oxidized flavor is presented. One class of substances which includes proteins, citrates, and amino acids inhibits by forming complex ions with copper, thus repressing its ionization.

Tyrosine, divalent manganese, lactose, and glucose inhibit oxidized flavor by being oxidized by hydrogen peroxide, thus protecting the phospholipides of milk.

ACKNOWLEDGMENT

The authors are indebted to Dr. R. B. Dustman, Department of Agricultural Chemistry, for his assistance and constructive criticisms in the preparation of this paper.

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THE EFFECT OF MACHINE MILKING UPON THE LEUCOCYTE COUNT AND THE CHLORIDE CONTENT OF MILK

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INTRODUCTION

In an earlier paper (2) it was shown that the leucocyte count and the chloride content of milk are not reliable indices of udder infection. Individual differences between cows with respect to these two tests and differences in results caused by factors other than infection frequently are greater than the changes that occur when the udder becomes infected. Any factor that causes udder irritation doubtless will cause a rise in the leucocyte and chloride values.

The extent to which machine milking may influence leucocyte counts and chloride determinations, or other mastitis tests that reflect udder irritation, such as pH and catalase tests, is not clear. Munch-Petersen (4) reviewed the literature on mastitis and found opinion about equally divided with respect to milking machines as a factor contributing to mastitis. Dahlberg (1) found that leaving machines on the cows too long contributed to udder troubles and resulted in a drop in production early in the lactation period. He presented data indicating that reducing to 4 or 5 minutes the time interval that the machines were left on the cows was accompanied by a drop in the leucocyte count of the mixed herd milk. Hucker (3) reported that the leucocyte count of milk from machine-milked cows was higher than that of milk from hand-milked cows. In his machine-milked group there was a higher incidence of streptococcic infections than in his hand-milked group, a factor which probably exaggerated the difference he found between the two groups with respect to leucocyte counts.

In studies on the effects of milking machines on tests for mastitis there has not to the author's knowledge been any report which deals separately with infected and uninfected cows. If milking machines cause irritation which predisposes the udder to infection, one should expect detectable increases in the leucocyte count and chloride content of the milk when a cow is on machine milking as compared with values found for the same cow when she is on hand milking. On the other hand, if the machine is only a fomite spreading the infection without itself predisposing the udder to the infection, high leucocyte counts and chloride values would be found in the milk of machine-milked cows because of the high incidence of infection and not directly as a result of injury caused by the machines. The purpose of this

Received for publication August 7, 1943.

paper is to report a study in which the effects of machine milking on cows are considered separately for infected and uninfected quarters.

METHODS

The data presented in this report were collected during the period from 1936 to 1942 in an investigation on mastitis in the Bureau of Dairy Industry herd at Beltsville, Maryland. The plan of the investigation was to begin with uninfected first calf heifers and continue them on the experiment as long as they remained in the herd. At intervals of 2 to 4 weeks individual quarter samples of the foremilk were taken from each cow and examined in the laboratory.

The examination consisted of the Hotis test, plating on Edwards aesculin crystal violet blood agar, a direct microscopic count of leucocytes and direct titration of chlorides with silver nitrate and a potassium chromate indicator. When there were abnormalities that could not be explained by the results on the Hotis test and by plating on the highly selective Edwards medium, the samples were plated in addition on standard tryptone glucose meat extract milk agar. Representative colonies were picked from the plates into meat infusion broth for identification of the organisms.

The herd at Beltsville is maintained primarily for experiments on feeding, breeding, and management. Changes from hand milking to machine milking or vice versa were made from time to time either in the interests of production records or for convenience in management. Usually when an animal was put on either hand or machine milking at the beginning of the lactation period, she remained on the same method of milking for the entire period. Occasionally, however, a change from one method of milking to the other was made during the lactation period. Under these circumstances it was possible to obtain data on 31 different cows that were on both methods of milking during the course of the investigation. For a few cows the data for a given method of milking extend over only parts of lactation periods, but for most of the cows one or more lactation periods were covered with each method of milking.

The data on the leucocyte counts and the chloride content for the different quarters have been divided into groups not only on the basis of the presence or absence of infection as determined by cultural tests, but also on the basis of the stage of lactation. For this purpose the lactation periods were divided into intervals of three months each. The data were also summarized for the entire lactation period.

THE EFFECT OF THE METHOD OF MILKING ON UNINFECTED QUARTERS

Table 1 shows the results obtained from the samples from the uninfected quarters of 27 cows. Of a total of 2,883 samples considered in this table, 1,571 were obtained while the cows were on hand milking and 1,312 while the cows were on machine milking.

TABLE 1

The effect of machine milking versus hand milking on the leucocyte count and chloride content of milk from uninfected quarters

Cow No.	First 3 months of lactation					
	Hand*			Machine*		
	Samples	Average chlorides	Average leucocytes	Samples	Average chlorides	Average leucocytes
	number	*per cent*	*thousands*	*number*	*per cent*	*thousands*
820	18	0.107	29	12	0.119	104
1235	6	0.128	82	15	0.186	804
1247	....	.....	.....	16	0.108	36
1265	....	.....	.....	20	0.099	26
1268	24	0.122	54	24	0.138	426
1269	13	0.110	287	35	0.113	269
1273	24	0.127	72	20	0.118	323
1274	20	0.111	31	14	0.102	235
1275	12	0.120	122	27	0.185	892
1278	16	0.124	52	10	0.111	310
1279	20	0.103	44	13	0.101	138
1282	32	0.105	24	24	0.130	488
1294	21	0.120	174	....	.....	.....
1298	12	0.111	48	28	0.128	119
1418	....	.....	.....	12	0.114	459
1434	33	0.120	77	....	.....	.....
1441	....	.....	.....	4	0.109	122
1445	16	0.094	26	47	0.090	188
1472	10	0.101	34	18	0.096	187
1473	10	0.088	22	32	0.110	917
1474	24	0.106	64	32	0.104	177
1476	16	0.116	159	19	0.104	1004
1478	....	.....	.....	9	0.097	302
1483	12	0.110	116	....	.....	.....
1488	15	0.089	23	21	0.099	372
1489	8	0.086	20	....	.....	.....
1490	14	0.090	229	21	0.094	90
Total or average†	376	0.110	78	473	0.116	365
	3 to 6 months in lacation					
820	15	0.117	20	15	0.132	257
1235	15	0.125	50	9	0.161	1051
1247	3	0.111	103	13	0.107	116
1265	....	.....	.....	24	0.106	99
1268	40	0.116	94	....	.....	.....
1269	15	0.102	200	28	0.117	186
1273	20	0.119	91	24	0.132	145
1274	16	0.114	75	28	0.106	170
1275	20	0.130	112	4	0.189	1402
1278	4	0.117	18	22	0.139	103
1279	....	.....	.....	18	0.102	128
1282	40	0.113	25	16	0.143	605
1294	34	0.119	88	12	0.120	369
1298	....	.....	.....	24	0.130	106
1418	....	.....	.....	....	.....	.....
1434	15	0.122	36	12	0.127	30
1441	....	.....	.....	24	0.116	339
1445	22	0.105	44	37	0.095	45
1472	10	0.103	65	....	.....	.....
1473	10	0.091	36	16	0.110	408
1474	20	0.116	306	36	0.119	637
1476	23	0.108	142	....	.....	.....
1478	....	.....	.....	22	0.101	151
1483	20	0.102	31	7	0.109	54
1488	16	0.096	23	8	0.091	169
1489	8	0.093	27	2	0.109	36
1490	....	.....	.....	18	0.101	318
Total or average†	366	0.113	83	419	0.117	258

* In the comparisons between hand and machine milking the data usually represent different lactation periods; in some instances, however, a cow was changed from one mode of milking to the other during a lactation period.

† Weighted average.

TABLE 1—(*Continued*)

Cow No.	Hand*			Machine*		
	Samples	Average chlorides	Average leucocytes	Samples	Average chlorides	Average leucocytes
	number	*per cent*	*thousands*	*number*	*per cent*	*thousands*
6 to 9 months in lactation						
820	12	0.126	54	9	0.186	383
1235	12	0.119	51	2	0.213	1505
1247	3	0.110	85	8	0.125	109
1265	14	0.134	190	4	0.121	45
1268	36	0.133	184	....	.....	.....
1269	15	0.109	31	9	0.128	51
1273	20	0.137	149	16	0.162	351
1274	32	0.112	60	4	0.127	471
1275	20	0.161	346	....	.....	.....
1278	....	.....	.....	16	0.159	385
1279	....	.....	.....	24	0.106	191
1282	40	0.119	20	4	0.240	2292
1294	6	0.111	76	8	0.136	439
1298	12	0.113	23	16	0.155	477
1418	....	.....	.....	4	0.097	429
1434	23	0.142	156	26	0.139	116
1441	9	0.106	73	4	0.128	571
1445	14	0.112	92	19	0.124	159
1472	12	0.101	27	4	0.110	95
1473	10	0.105	388	8	0.112	365
1474	20	0.123	208	25	0.141	991
1476	30	0.107	116	....	.....	.....
1478	....	.....	.....	20	0.107	148
1483	16	0.107	69	12	0.124	165
1488	22	0.102	44	....	.....	.....
1489	6	0.102	18	8	0.107	36
1490	....	.....	.....	6	0.123	239
Total or average†	384	0.120	115	256	0.134	352
9 to 12 months in lactation						
820	21	0.139	409	6	0.167	317
1235	19	0.161	235	....	.....	.....
1247	20	0.129	761	....	.....	.....
1265	20	0.145	53	....	.....	.....
1268	24	0.149	571	....	.....	.....
1269	18	0.122	54	5	0.121	265
1273	24	0.165	165	11	0.270	4518
1274	38	0.113	47	....	.....	.....
1275	12	0.190	676	....	.....	.....
1278	....	.....	.....	....	.....	.....
1279	....	.....	.....	16	0.141	809
1282	36	0.139	81	....	.....	.....
1294	....	.....	.....	....	.....	.....
1298	24	0.119	22	....	.....	.....
1418	16	0.099	61	3	0.108	103
1434	3	0.135	151	29	0.149	361
1441	....	.....	.....	....	.....	.....
1445	17	0.109	188	2	0.177	1748
1472	8	0.107	36	7	0.107	39
1473	10	0.104	45	5	0.095	40
1474	20	0.122	161	8	0.183	1280
1476	30	0.115	105	....	.....	.....
1478	8	0.104	52	16	0.126	257
1483	12	0.119	104	12	0.130	218
1488	14	0.106	97	....	.....	.....
1489	10	0.116	18	8	0.110	38
1490	16	0.100	20	....	.....	.....
Total or average†	420	0.128	150	128	0.148	643

* In the comparisons between hand and machine milking the data usually represent different lactation periods; in some instances, however, a cow was changed from one mode of milking to the other during a lactation period.

† Weighted average.

TABLE 1—(*Continued*)

Cow No.	More than 12 months in lactation					
	Hand*			Machine*		
	Samples	Average chlorides	Average leucocytes	Samples	Average chlorides	Average leucocytes
	number	*per cent*	*thousands*	*number*	*per cent*	*thousands*
820	.....	.....	.....	15	0.178	1204
1235	.....	.....	.....	.....	.....	.....
1247	.....	.....	.....	8	0.177	859
1265	.....	.....	.....	.....	.....	.....
1268	.....	.....	.....	.....	.....	.....
1269	21	0.157	451	.....	.....	.....
1273	.....	.....	.....	.....	.....	.....
1274	.....	.....	.....	.....	.....	.....
1275	.....	.....	.....	.....	.....	.....
1278	.....	.....	.....	.....	.....	.....
1279	.....	.....	.....	.....	.....	.....
1282	.....	.....	.....	.....	.....	.....
1294	.....	.....	.....	.....	.....	.....
1298	.....	.....	.....	.....	.....	.....
1418	.....	.....	.....	.....	.....	.....
1434	.....	.....	.....	.....	.....	.....
1441	.....	.....	.....	.....	.....	.....
1445	.....	.....	.....	.....	.....	.....
1472	.....	.....	.....	9	0.142	328
1473	.....	.....	.....	.....	.....	.....
1474	.....	.....	.....	.....	.....	.....
1476	.....	.....	.....	.....	.....	.....
1478	.....	.....	.....	.....	.....	.....
1483	.....	.....	.....	.....	.....	.....
1488	.....	.....	.....	.....	.....	.....
1489	.....	.....	.....	.....	.....	.....
1490	4	0.098	18	4	0.104	435
Total or average†	25	0.147	382	36	0.163	823
	Summary for entire period					
820	66	0.123	153	57	0.153	500
1235	52	0.137	121	26	0.180	885
1247	26	0.125	80	45	0.122	218
1265	34	0.140	110	48	0.104	64
1268	124	0.129	205	24	0.138	426
1269	82	0.123	215	77	0.117	213
1273	88	0.138	119	71	0.156	700
1274	106	0.112	52	46	0.106	216
1275	64	0.148	293	31	0.186	958
1278	20	0.122	45	48	0.140	240
1279	20	0.103	44	71	0.112	305
1282	148	0.119	37	44	0.145	695
1294	61	0.119	117	20	0.126	397
1298	48	0.116	29	68	0.135	199
1418	16	0.099	61	19	0.110	397
1434	74	0.128	96	67	0.141	206
1441	9	0.106	73	32	0.117	341
1445	69	0.105	85	105	0.101	163
1472	40	0.103	40	38	0.110	184
1473	40	0.097	123	61	0.109	639
1474	84	0.116	179	101	0.125	630
1476	99	0.111	126	19	0.104	1004
1478	8	0.104	52	67	0.108	196
1483	60	0.108	73	31	0.123	160
1488	67	0.098	45	29	0.097	316
1489	32	0.100	21	18	0.108	37
1490	34	0.096	106	49	0.103	225
Total or average†	1571	0.118	112	1312	0.124	368

* In the comparisons between hand and machine milking the data usually represent different lactation periods; in some instances, however, a cow was changed from one mode of milking to the other during a lactation period.

† Weighted average.

The figures at the bottom of table 1, giving average results for all of the cows in this group, show that in every stage of lactation machine milking resulted in higher leucocyte counts and chloride values than did hand milk-

TABLE 2

The effect of machine milking versus hand milking on the leucocyte count and chloride content of milk from infected quarters

Cow No.	Infecting organism	First 3 months of lactation					
		Hand*			Machine*		
		Samples	Average chlorides	Average leucocytes	Samples	Average chlorides	Average leucocytes
		number	*per cent*	*thousands*	*number*	*per cent*	*thousands*
840	*Streptococcus*	15	0.168	2931	9	0.169	4331
859	*Streptococcus*	.....	.....	.....	8	0.175	4984
882	*Streptococcus*	12	0.182	5823	.....	.....	.....
1235	*Streptococcus*	5	0.150	5730	13	0.235	5083
1269	*Ps. aeruginosa*	7	0.118	3375	4	0.148	3822
1445	*Streptococcus*	11	0.114	2288	11	0.138	4290
1472	*Streptococcus*	10	0.121	573	6	0.116	1692
1473	*Ps. aeruginosa*	10	0.122	1230	11	0.178	9355
1479	*Ps. aeruginosa*	8	0.093	1860	.....	.....	.....
Total or average†		78	0.137	2874	62	0.173	5169
		3 to 6 months in lactation					
840	*Streptococcus*	6	0.175	1901	21	0.171	2895
859	*Streptococcus*	2	0.211	5140	14	0.180	5668
882	*Streptococcus*	6	0.176	1702	6	0.199	8157
1235	*Streptococcus*	5	0.135	141	19	0.180	4889
1269	*Ps. aeruginosa*	5	0.113	5022	2	0.159	17500
1445	*Streptococcus*	15	0.121	940	9	0.158	2310
1472	*Streptococcus*	10	0.125	370	20	0.120	2524
1473	*Ps. aeruginosa*	10	0.133	6337	23	0.170	10329
1479	*Ps. aeruginosa*	7	0.101	785	2	0.133	2975
Total or average†		66	0.134	2188	116	0.164	5446
		6 to 9 months in lactation					
840	*Streptococcus*	3	0.177	1129	12	0.209	5723
859	*Streptococcus*	13	0.127	1031	3	0.207	3273
882	*Streptococcus*	15	0.142	2054	.....	.....	.....
1235	*Streptococcus*	4	0.118	77	6	0.245	6407
1269	*Ps. aeruginosa*	5	0.108	801	5	0.153	8150
1445	*Streptococcus*	8	0.139	562	6	0.215	2521
1472	*Streptococcus*	12	0.137	1065	16	0.127	3519
1473	*Ps. aeruginosa*	10	0.166	7370	18	0.135	12346
1479	*Ps. aeruginosa*	6	0.112	2625	8	0.141	2754
Total or average†		76	0.144	2142	74	0.166	6397

* In the comparisons between hand and machine milking the data usually represent different lactation periods; in some instances, however, a cow was changed from one mode of milking to the other during a lactation period.

† Weighted average.

TABLE 2—(*Continued*)

Cow No.	Infecting organism	More than 9 months in lactation					
		Hand*			Machine*		
		Samples	Average chlorides	Average leucocytes	Samples	Average chlorides	Average leucocytes
		number	*per cent*	*thousands*	*number*	*per cent*	*thousands*
840	*Streptococcus*	.....	.....	.....	3	0.181	2276
859	*Streptococcus*	6	0.166	1215	.....	.....	.....
882	*Streptococcus*	6	0.207	2701	.....	.....	.....
1235	*Streptococcus*	6	0.157	321	.....	.....	.....
1269	*Ps. aeruginosa*	13	0.164	9548	17	0.145	4511
1445	*Streptococcus*	4	0.176	6417	.....	.....	.....
1472	*Streptococcus*	18	0.146	1219	18	0.120	1479
1473	*Ps. aeruginosa*	20	0.144	6850	.....	.....	.....
1479	*Ps. aeruginosa*	6	0.153	8531	8	0.180	6692
Total or average†		79	0.158	4877	46	0.144	3558
		Summary for entire period					
840	*Streptococcus*	24	0.171	2448	45	0.181	3895
859	*Streptococcus*	21	0.170	1671	25	0.181	5162
882	*Streptococcus*	39	0.169	3259	6	0.199	8157
1235	*Streptococcus*	20	0.142	1577	38	0.209	5195
1269	*Ps. aeruginosa*	30	0.136	5896	28	0.148	5990
1445	*Streptococcus*	38	0.129	1827	26	0.163	3196
1472	*Streptococcus*	50	0.134	883	60	0.121	2392
1473	*Ps. aeruginosa*	50	0.142	5727	52	0.160	10821
1479	*Ps. aeruginosa*	27	0.113	3234	18	0.157	4529
Total or average†		299	0.144	3066	298	0.163	5333

* In the comparisons between hand and machine milking the data usually represent different lactation periods; in some instances, however, a cow was changed from one mode of milking to the other during a lactation period.

† Weighted average.

ing. With some exceptions the results for each individual cow also show higher leucocyte and chloride levels when milking machines were used. With cow 1,269 the differences are so small as to be insignificant. On the other hand, certain cows, such as 1,235 and 1,275, show much higher results on machine milking than on hand milking. The results on cows such as those two tend to exaggerate the group average figures for machine milking.

Summary figures on cow 1,265, shown in the section on the extreme right of table 1, indicate that the leucocytes and chlorides in the milk were lower while she was on machine milking than they were while she was on hand milking. The figures for the different stages of lactation show, however, that for 1,265 all of the results on machine milking were obtained during the first six months of lactation and all of the results on hand milking were obtained during the last six months of lactation. That lack of direct comparison possibly accounts for the apparently lower results with machine

milking. This surmise is borne out by the group average figures at the bottom of table 1, which show that with both methods of milking there is a steady increase in leucocytes and chlorides as lactation advances.

THE EFFECT OF THE METHOD OF MILKING ON INFECTED QUARTERS

Table 2 shows the results for the infected quarters of 9 cows that were on both hand and machine milking after the onset of infection. Of a total of 597 samples reported here, 299 were taken while the cows were on hand milking and 298 while they were on machine milking.

As would be expected, the leucocyte counts and chloride values in these samples were distinctly higher than those reported in table 1 for the samples from uninfected quarters. The variations found in table 2 are greater than those found in table 1 and there is less tendency with these infected quarters for the leucocytes and chlorides to rise with advancing lactation.

In general, the results in table 2 indicate that with infected quarters, as with uninfected quarters, the leucocyte counts and chloride values are higher when the cows are on machine milking than when the cows are on hand milking.

RELATION OF THE METHOD OF MILKING TO THE OCCURRENCE OF INFECTION

Whenever a newly infected quarter was found during this investigation an attempt was made to correlate the onset of the infection with the method of milking that was being used at the time. Infections that occurred during the dry period and a few infections that occurred at about the time a cow was changed from one method of milking to the other were not ascribed to either method of milking.

Of 23 streptococcic infections, the onset of which could be correlated with the method of milking, 9 occurred in cows on hand milking and 14 in cows on machine milking. During the period that these infections occurred, the numbers of cows on the two methods of milking were approximately the same. Since the total number of traceable cases was small, it is doubtful whether the difference between the 9 cases occurring on hand milking and the 14 cases occurring on machine milking is significant.

On the other hand, infections with *Pseudomonas aeruginosa* are rather definitely associated with machine milking. The onset in 29 cases of *Ps. aeruginosa* can definitely be traced to periods when the affected cows were on machine milking. Only one case has been found that appears to have originated when the cow was on hand milking. The evidence with respect to several doubtful cases is that they, also, probably are associated with machine milking.

Infections associated with organisms other than streptococci and *Ps. aeruginosa* were too few to be of significance in this study.

DISCUSSION

At the time this investigation was under way little attention was given to the length of time that the machines were left operating on the cow. The general practice was to remove the teat cups as soon as milk flow ceased, but when it was known that a cow was not letting her milk down, the machine was left on the cow for an indefinitely longer period in an effort to accomplish complete milking. According to Petersen (5) this practice will ultimately make the cow a stripper. Petersen further asserts that to leave the machine on the cow after the milk has ceased flowing may injure the lining of the teat cistern.

Dahlberg (1) has shown that cows on machine milking remain in high production longer and give milk with fewer leucocytes if the machines are left on the cow a maximum of 4 or 5 minutes at a milking. Some of the manufacturers of milking machines are now recommending an interval of milking time not to exceed 5 minutes, insisting that slow milking cows can be trained to fast milking with benefit to both production and the health of the udder.

It is quite probable that if, during this investigation, the practice now recommended for limiting milking time to 5 minutes had been adhered to, the difference in results between hand and machine milking would have been less than was actually found. Just how much less is purely conjecture. It is apparent that under the conditions of this experiment the use of milking machines caused some udder irritation and contributed significantly to the spread of *Ps. aeruginosa* infections. The relationship between the use of milking machines and the incidence of streptococcic infections in the herd is not definite.

SUMMARY AND CONCLUSIONS

The effect of milking machines on cows has been studied by comparing the results of leucocyte counts and chloride titrations of the milk when the cows were on machine milking with the results found when the same cows were on hand milking. Data for infected and uninfected quarters were studied separately. A total of 31 cows was included in the two groups.

Under the conditions of this experiment the milk of most of the cows when on machine milking yielded higher leucocyte and chloride values than when they were on hand milking. This was true for both infected and uninfected quarters. There were marked differences between cows in their response to machine milking.

Milking machines apparently contributed to the spread of *Ps. aeruginosa* infections in the herd. Whether they were a factor in the spread of streptococcic infections is not definite.

The influence of the interval of time that the machines were left on the cows as a factor in causing the high leucocyte and chloride levels with machine milking is discussed.

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AMMONIATED SUGAR BEET PULP AS A NEW NITROGENOUS FEED FOR RUMINANTS

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It has been shown (9, 10) that sugar beet pulp can be easily ammoniated to produce a palatable product containing 4 to 5 per cent nitrogen. The added nitrogen is stable and mostly in a water-soluble form. Studies (11) on pilot plant ammoniation of the dried pulp indicate that commercial ammoniation would be a relatively simple and inexpensive process. Since considerable evidence (3, 4, 5, 13) has accumulated to show that ruminants can use urea and ammonium bicarbonate for their maintenance and growth it seemed desirable to learn whether ruminants can also use ammoniated pulp for their nitrogenous nutrition. The experimental work reported in this paper was designed to give information on that point.

The term ammoniated pulp as used in this paper refers to a plain dried sugar beet pulp which has been treated with ammonia (11) to raise its nitrogen content. Likewise, ammoniated molasses pulp refers to a molasses beet pulp which has been treated with ammonia to increase its nitrogen content. Molasses pulp is a product usually made by mixing molasses with wet beet pulp at the beginning of the drying process.

On May 12, seven Holstein male calves weighing 125 to 187 pounds were placed on a milk and calf meal diet. These animals developed scours soon thereafter which held them back somewhat. They were fed milk, calf meal, alfalfa and molasses beet pulp from May 12 to July 26 with the animals receiving little milk the last month.

At the beginning of the experimental period the animals were placed in separate specially prepared pens inside an enclosed hay barn. Each pen was further protected during the coldest part of the winter and liberal amounts of pine shavings were used as bedding. Even so, it was very cold in these pens during the subzero weather. The animals were allowed to run in a vegetation-free paddock when the weather would permit.

The ammoniated pulps were prepared by adding 300 pounds of pulp to the closed ammoniation unit. Fifteen pounds of ammonia were added and the unit was revolved for thirty minutes. The temperature became 130° C. and a product containing 25–27 per cent protein ($N \times 6.25$) was obtained.

Received for publication August 7, 1943.

[1] The author gratefully acknowledges the aid of Mr. Fred Rath for the care of the animals; of Mr. D. A. Greenwood for his many suggestions and aid in securing the blood; of Mr. R. C. Jenkins of the Moore Clinical Laboratories, of Dr. J. S. Bengston, Pathological Laboratories, U. S. Bureau of Meat Inspection, for the examination of the liver and kidneys and to Arline Lilly and Betty Soderstrom for analytical help.

Unammoniated pulp was then mixed with the ammoniated to give the necessary protein value for the rations.

On July 26, the animals were placed on the experimental rations shown in table 1 and each animal was referred to by the ration he ate. The rations were essentially alike except for the nitrogen source and the fact that starch was substituted for nitrogen free molasses in some cases.

TABLE 1

The composition of the basal ration

Ingredients		Protein	
	lbs.	%	*lbs.*
Plain dried beet pulp	60.00	9.2	5.52
Timothy hay	16.00	8.0	1.28
Starch	8.79	0.0	0.00
Molasses	12.00	0.0	0.00
Steamed bone meal	2.00	7.0	0.14
Iodized salt	1.00	0.0	0.00
Fortified fish oils	0.21	0.0	0.00
	100.00	6.94	6.94

Calves 1 and 2 received the basal ration. This contained 6.94 protein (N × 6.25). Rations 1 and 2.

Calf 3 received the basal ration modified by substituting ammoniated pulp for plain pulp and starch for molasses. Total protein (N × 6.25) was 12.42 per cent. Ration 3.

Calf 4 received the basal ration modified by substituting ammoniated pulp for plain pulp. Total protein (N × 6.25) was 12.42 per cent. Ration 4.

Calf 5 received the basal ration modified by substituting ammoniated pulp for plain pulp and starch for molasses. Total protein (N × 6.25) was 17.02 per cent. Ration 5.

Calf 6 received the basal ration modified by substituting ammoniated pulp for plain pulp. The protein (N × 6.25) was 17.02 per cent. Ration 6.

Calf 7 received the basal ration except toasted soybean meal was substituted for the starch and molasses. Total protein (N × 6.25) was 16.42 per cent. Ration 7.

Calf 8 received the basal ration except molasses ammoniated pulp was substituted for plain pulp. Total protein (N × 6.25) was 17.02 per cent. Ration 8.

Molasses was excluded from rations 3 and 5 to note whether this soluble carbohydrate promoted the use of the nitrogen in the ammoniated pulp.

The calves were fed equal amounts of feed three times daily and sufficient fortified fish oils were added to each feeding to supply each animal daily with 2700 units of vitamin A and 500 units of vitamin D per 100 pounds of body weight. Individual weights were taken weekly

during the experimental periods and the increase was recorded as growth. The animals were weighed daily the last three days and the mean was taken as the final value.

Pictures were taken of the animals 132 and 222 days from the beginning of the experiment. Calves 1 and 2 were taken from the basal diet and placed on rations 8 and 5 respectively, 150 days from the beginning of the experiment. The second pictures were taken 72 days after the protein deficient animals had been changed to diets containing ammoniated pulp.

Blood was taken from the jugular vein of each animal 97, 140 and 210 days after the beginning of the experiment. It was analyzed for total erythrocytes, hemoglobin, total leucocytes, neutrophiles, eosinophiles, monocytes and lymphocytes by the usual clinical methods. The color index was then calculated by the usual procedure. The amounts of glucose, calcium, total serum protein, non-protein nitrogen, urea, cholesterol, serum albumin and serum globulin were determined (6, 7) and the albumin-globulin ratio was calculated.

The steers were slaughtered at the Swift and Company yards under federal inspection and the liver from No. 7 and a kidney from each animal were sent to the pathological laboratory for further investigation.

The animal tissues were analyzed (1) for total protein, water-soluble protein, coagulable protein and moisture. The A.O.A.C. method was modified for the water-soluble and coagulable-proteins on the livers and kidneys as follows:

Ten to thirteen grams of freshly ground sample was exhausted with 10 cc. of water at room temperature. The sample was transferred to a 250-cc. volumetric flask with water and diluted almost to the mark and stored in the refrigerator 65 hours at 2° C. Samples were removed from the refrigerator in pairs and made up to volume immediately. They were mixed well and the entire 250 cc. was centrifuged thirty minutes. The supernatant portion was decanted and 25-cc. room-temperature aliquots were used for water-soluble proteins and 75-cc. for coagulable proteins. One cc. of 0.1 N acetic acid was added to the 75 cc. and the sample was boiled five minutes to coagulate the proteins. The coagulum was transferred to a filter and washed with 250 cc. of hot water for the livers and 25 cc. hot water for the kidneys. Nitrogen was determined by the Kjeldahl method and protein was calculated ($N \times 6.25$).

EXPERIMENTAL RESULTS

It was not necessary to work the animals on to these feeds for the palatability was such that they ate well from the beginning except for the first animal on ration 6. He did not clean his feed up readily until he was placed upon ration 4 later in the experiment. Each animal consumed 5 pounds of feed early in the experiment. This was increased from time to

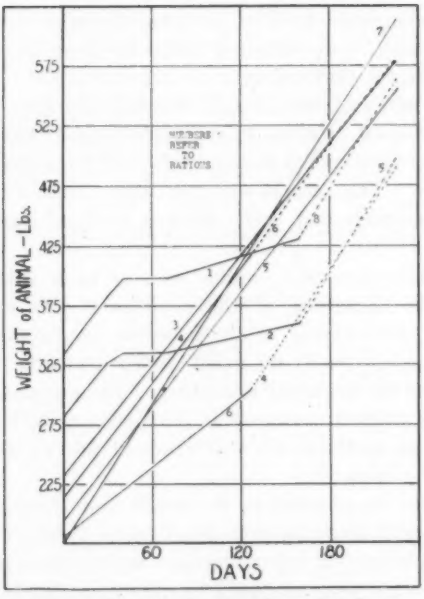

Fig. 1. Growth of male calves—225 days.

time until after 34 days 12 pounds were being eaten. After 70 days the amount was increased to 15 pounds. This proved to be too much so the amount was decreased to 11 pounds for the rest of the experiment. The

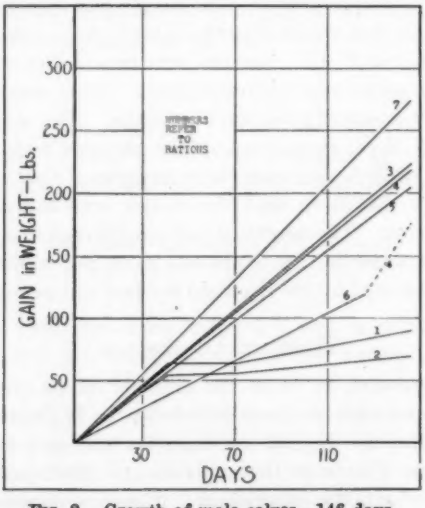

Fig. 2. Growth of male calves—146 days.

appetites were good and equalized feeding was practiced throughout without difficulty. No digestion troubles of any kind developed. There was no evidence of diuresis in any of the animals. The growth of the animals is shown in figures 1, 2 and 3. Figure 1 shows the weight of the animals during the experiment (225 days). Figure 2 shows the gain in weight after 146 days and figure 3 shows the gain in weight at the end of 225 days.

The animals on rations 1 and 2 continued to grow quite rapidly during the first 40 days of the ration but began to develop protein deficiency symptoms

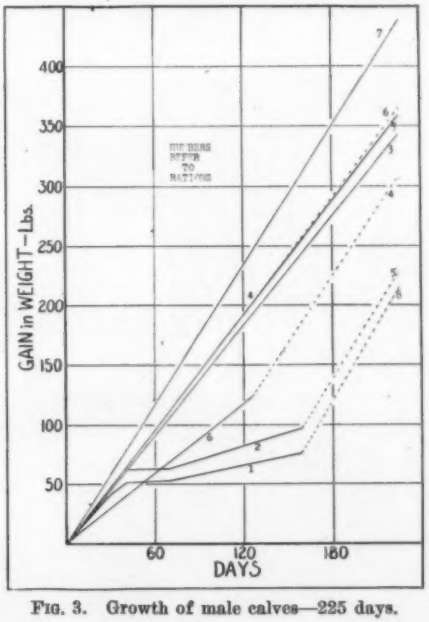

FIG. 3. Growth of male calves—225 days.

at the end of 30 days. Then they did not grow at all for 30 days, after which they grew very slowly until they were placed on rations 8 and 5, respectively.

The protein deficiency symptoms as shown in figure 4 became very marked before they were placed on the rations containing ammoniated pulp. The animals became very thin, developed a sharp-boned hump in their back, a pot belly, a rough and shaggy coat and a listless gait.

After the protein-deficient animals were placed on rations 8 and 5 they started to lose their protuberant bellies, their backbones straightened out, their coats took on more life, they became much more lively, and began to grow at the rate of about 2 pounds per day.

There was little difference in the growth rates of animals 3, 4 and 5. Molasses was excluded from rations 3 and 5 to note whether the absence of

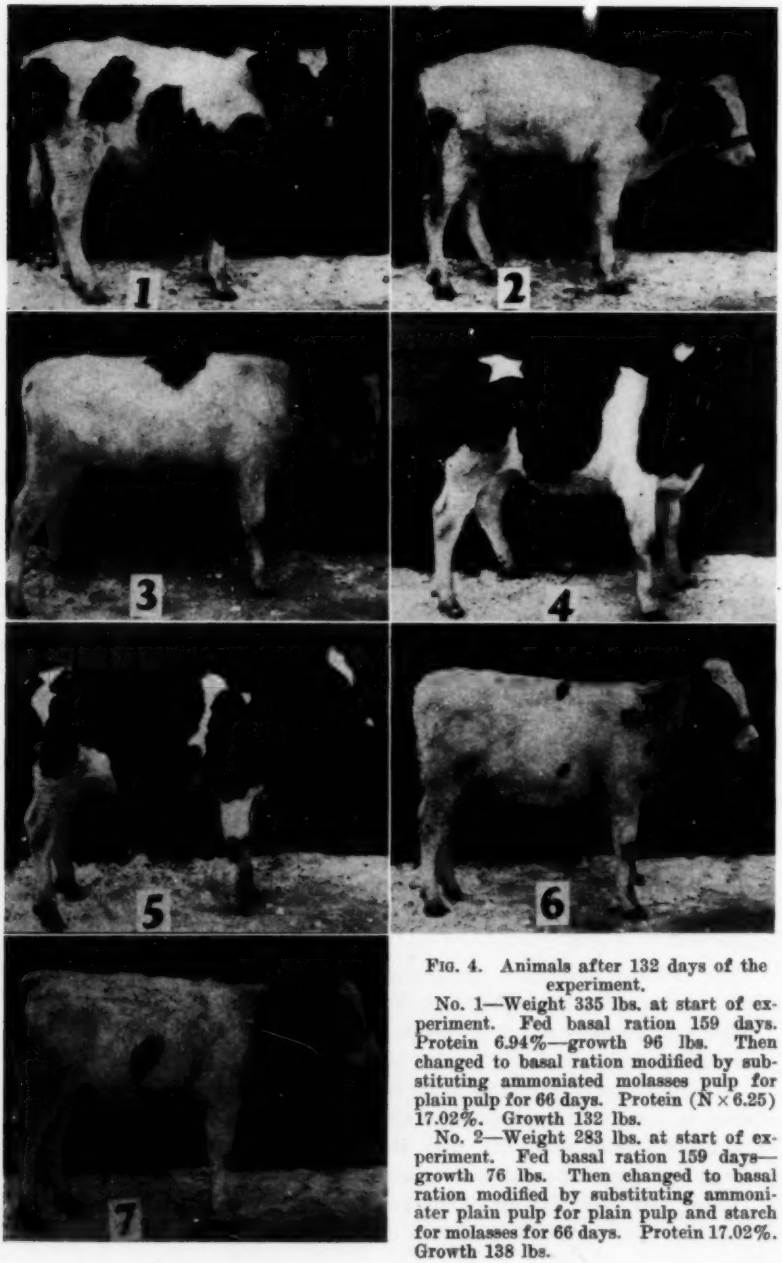

FIG. 4. Animals after 132 days of the experiment.

No. 1—Weight 335 lbs. at start of experiment. Fed basal ration 159 days. Protein 6.94%—growth 96 lbs. Then changed to basal ration modified by substituting ammoniated molasses pulp for plain pulp for 66 days. Protein (N × 6.25) 17.02%. Growth 132 lbs.

No. 2—Weight 283 lbs. at start of experiment. Fed basal ration 159 days—growth 76 lbs. Then changed to basal ration modified by substituting ammoniater plain pulp for plain pulp and starch for molasses for 66 days. Protein 17.02%. Growth 138 lbs.

this source of soluble carbohydrate inhibited the use of the nitrogen in the ammoniated pulp or conversely whether its presence promoted nitrogen utilization over that of starch alone. The results indicate no difference in the growth of the animals on rations containing all starch as compared to those in which molasses replaced part of the starch.

The animal on ration 6 grew very slowly during the preliminary 14 weeks before the experiment was started. Furthermore, he continued to grow more slowly than animals 3, 4, 5 and 7 from the beginning of the experiment. This animal was on the ration containing molasses and pulp ammoniated to 4.16 per cent nitrogen. He did not eat it as well as the other animals were eating their rations. Therefore, after 159 days the rations of animals 4 and 6 were exchanged to learn if there was something about ration 6 to give the inferior results. After this the slow growing animal grew much faster on ration 4 while the new animal on ration 6 continued to eat and grow at its previous rate on ration 4. Apparently it was not the ration alone but probably an animal-ration relationship causing the poor initial results from ration 6. Growth was just as good on rations containing 12.42 per cent protein as on those containing 17.02 per cent protein.

The pictures of the animals at the end of the experiment are shown in figure 5. They show that the animals on ammoniated pulp grew during the 90-day interval between pictures and that the physical condition of the protein deficient animals was greatly improved by the ammoniated pulp.

The data in table 2 show the daily gains and feed consumed per 100 pounds gain in weight for the various growing periods shown in figure 1. The soybean fed animal gained 1.956 pounds per day for 225 days. Ration one provided only 14.26 per cent of this growth for animal 1; ration two, 10.38 per cent; ration three, 78.07 per cent; ration four, 81.80 and 95.55 per cent; ration five, 81.34 and 106.90 per cent and ration six, 49.89 and 84.15 per cent. The larger value on ration 5 was obtained when it was fed to animal 2 that had been on the protein deficient diet. Animal 4 did about equally well on rations 4 or 6 while animal 6 grew much better on ration 4 than ration 6. The feed consumed per 100-pound gain as compared to those listed by Morrison (14) must be considered good.

No. 3—Initial weight 234 lbs. Fed basal ration modified by substituting ammoniated plain pulp for plain pulp and starch for molasses. Protein 12.42%. Growth after 159 days 244 lbs.; after 225 days 343 lbs.

No. 4—Initial weight 214 lbs. Fed basal ration modified by substituting ammoniated plain pulp for plain pulp. Protein 12.42%. Growth after 159 days 261 lbs.; after 225 days 364 lbs.

No. 5—Initial weight 195 lbs. Fed basal ration modified by substituting ammoniated plain pulp for plain pulp and starch for molasses. Protein 17.02%. Growth after 159 days 255 lbs.; after 225 days 358 lbs.

No. 6—Initial weight 181 lbs. Fed basal ration modified by substituting ammoniated pulp for plain pulp for 126 days. Protein 17.02%. Growth 123 lbs. Then changed to ration four for 99 days. Growth 185 lbs.

No. 7—Initial weight 174 lbs. Fed basal ration modified by substituting toasted soybean meal for starch. Protein 16.42%. Growth after 159 days 313 lbs.; after 225 days 440 lbs.

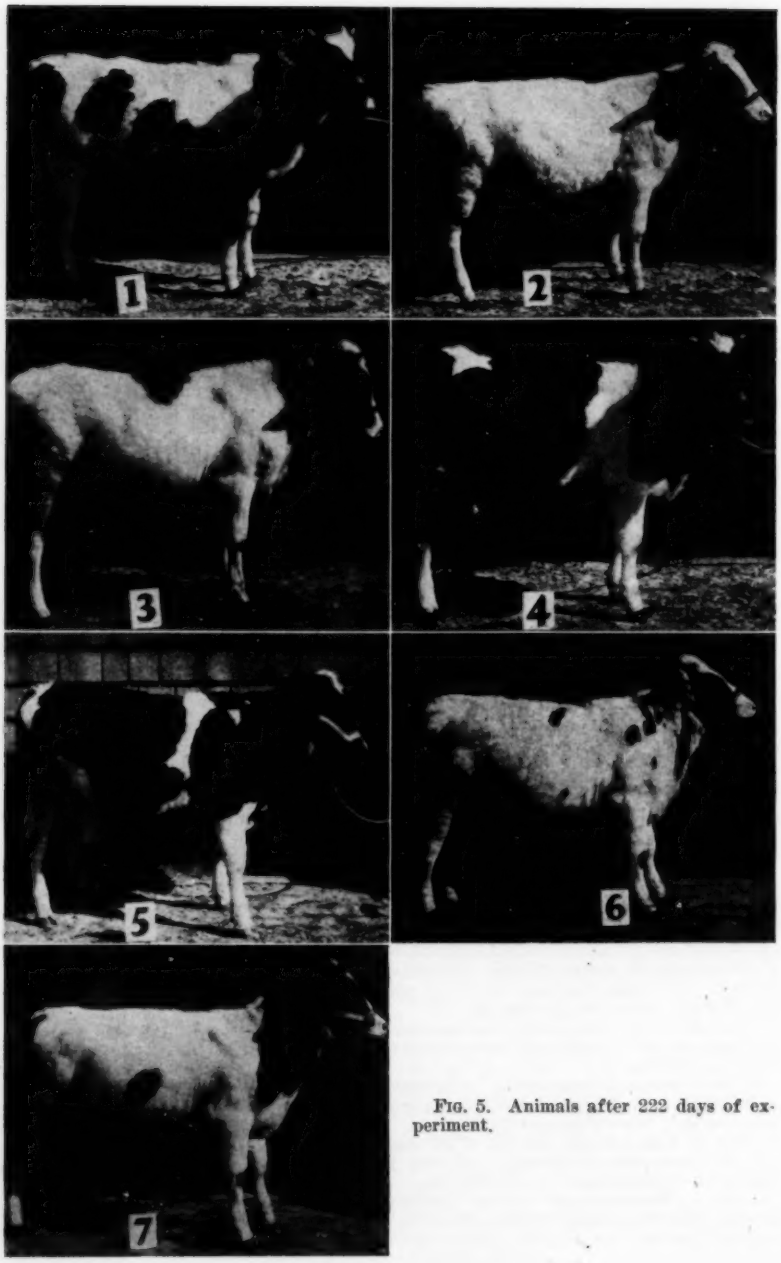

FIG. 5. Animals after 222 days of experiment.

ANALYSES OF THE BLOOD

The data on the blood are shown in table 3. The first two blood samplings were taken while animals 1 and 2 were still on the deficient rations. The third samples were taken near the end of the experiment and after animals 1 and 2 had been changed to the ammoniated rations and had gained rapidly in weight. Thus, blood from animals on ammoniated pulp was obtained from the third sampling of animals 1 and 2 and from all three samplings from animals 3, 4, 5 and 6.

The blood from animal No. 7 represents that from an animal on a conventional protein source. The approximate normal value (2) for cattle

TABLE 2

Rate of gain and amount of feed consumed by steers during certain feeding periods

Steer No.	Ration No.	Experimental period	Daily gain	Portion of daily gain promoted by soybean meal	Total feed consumed	Feed consumed per 100 lbs. body weight
		days	*lbs.*	*%*	*lbs.*	*lbs.*
1	Basal	41–159	0.279	14.26	1463	4433
	Ration 8	159–225	2.000	102.25	726	550
2	Basal	41–159	0.203	10.38	1463	6096
	Ration 5	159–225	2.091	106.90	726	526
3	Ration 3	1–225	1.527	78.07	2545	742
4	Ration 4	1–126	1.600	81.80	1456	728
	Ration 6	126–225	1.646	84.15	1089	667
5	Ration 5	1–225	1.591	81.34	2545	711
6	Ration 6	1–126	0.976	49.89	1456	1184
	Ration 4	126–225	1.869	95.55	1089	589
7	Ration 7 (Soybean meal)	1–225	1.956	100.00	2545	579

listed in table 3 represents mean values and are largely based on adult cattle. Miller (12) has shown that the number of erythrocytes decreases in calves as they mature while the hemoglobin values remain quite constant with wide differences in individual animals. He shows that calves appear to have more leucocytes than the adult with the proportions of the kinds of leucocytes remaining about the same as in adults. He also shows that young bovine animals have less serum protein than adults. Hodgson *et al.* (8) show that the glucose in the blood of dairy animals expressed in milligrams per 100 cc. of blood was 100.4, 88.2, 80.2, 75.4, 69.6, 67.8, 62.2 and 55.0 after 6 days, 4 weeks, 3, 7, 11, 15, 19 and 23 months, respectively.

The data show the total erythrocytes to be normal in all cases. The third sample from animal No. 7 was somewhat higher than the other values. The hemoglobin was normal in all samplings and the total leucocytes were about the same except that the first two samplings on the soybean-meal animal were high. Perhaps the abnormal leucocyte and erythrocyte values on the soy-

TABLE 3

Composition of blood of growing Holstein steers

Steer No.	Ration No.	Blood sampling date	Erythrocytes		Color index	Leucocytes				
			Total count per cu. mm.	Hemoglobin		Total count per cu. mm.	Neutrophiles (adults)	Eosinophiles	Monocytes	Lymphocytes
				gms./100 cc.			*per cent*	*per cent*	*per cent*	*per cent*
1	1	1*	7,450,000	9.7	0.42	12,700	14	4	.....	82
	1	2	8,560,000	9.8	0.37	12,000	29	1	.....	70
	8	3	7,930,000	9.7	0.40	8,500	23	.....	.....	77
2	2	1	7,550,000	9.7	0.40	6,600	26	1	1	72
	2	2	9,240,000	9.7	0.34	6,000	18	1	.....	81
	5	3	7,810,000	9.2	0.40	9,300	11	1	2	86
3	3	1	8,850,000	12.2	0.45	8,800	27	.....	1	73
	3	2	7,410,000	10.7	0.47	11,050	24	4	1	70
	3	3	7,600,000	9.2	0.40	10,700	29	.....	.....	70
4	4	1	9,630,000	10.6	0.36	8,700	34	1	1	64
	6	2	8,480,000	9.2	0.36	7,800	27	.....	.....	73
	6	3	8,700,000	10.7	0.40	12,200	27	.....	.....	73
5	5	1	9,600,000	10.3	0.35	4,800	23	1	2	64
	5	2	9,190,000	10.7	0.35	7,200	24	2	.....	74
	5	3	8,840,000	10.7	0.39	7,900	28	.....	.....	72
6	6	1	9,650,000	11.2	0.40	6,300	27	2	.....	71
	4	2	9,370,000	9.8	0.34	8,250	22	1	1	76
	4	3	9,100,000	10.6	0.37	13,700	31	.....	.....	69
7	7	1	7,500,000	9.4	0.40	23,900	25	.....	1	74
	7	2	8,630,000	9.9	0.32	22,200	27	3	.....	70
	7	3	11,560,000	11.7	0.33	10,100	46	.....	.....	54
Approximate normal for cattle (2)			6,325,000	10.3	.....	7,900†	21	5	10	64

TABLE 3—(*Continued*)

Steer No.	Ration No.	Blood sampling date	Glucose	Calcium	Cholesterol	Non-protein nitrogen	Urea N	Total serum protein	Serum		Albumin/globulin ratio
									Albumin	Globulin	
			mgms./100 cc. blood	*mgms./100 cc. serum*	*mgms./100 cc. serum*	*mgms./100 cc. blood*	*mgms./100 cc. blood*	*per cent*			
1	1	1*	74.0	11.51	88	18.0	11.0	6.56	3.94	2.62	1.50/1
	1	2	69.5	9.70	127	26.9	9.2	5.60	3.51	2.09	1.68/1
	8	3	83.0	10.00	81	32.0	15.4	4.38	2.48	1.90	1.31/1
2	2	1	73.0	12.93	58	16.0	7.8	7.88	4.28	3.60	1.19/1
	2	2	74.5	10.70	121	.....	7.1	5.66	3.51	2.15	1.63/1
	5	3	94.0	10.60	62	33.5	11.2	5.05	3.00	2.05	1.46/1
3	3	1	45.0	12.73	44	26.0	9.2	7.98	4.52	3.46	1.31/1
	3	2	82.0	10.90	101	34.1	9.3	5.49	3.85	1.64	2.34/1
	3	3	89.0	10.50	69	32.0	8.1	5.22	3.55	1.67	2.13/1
4	4	1	77.0	12.42	55	24.8	9.0	5.49	3.94	1.55	2.54/1
	6	2	77.5	11.10	85	33.3	10.0	4.76	3.32	1.44	2.31/1
	6	3	97.0	11.10	71	29.5	9.1	6.42	3.42	3.00	1.14/1
5	5	1	65.0	11.31	58	30.0	16.3	6.18	4.10	2.08	1.97/1
	5	2	81.0	10.60	81	26.5	15.1	5.45	4.15	1.30	3.19/1
	5	3	89.0	9.60	72	32.0	16.0	5.33	3.45	1.88	1.84/1
6	6	1	55.0	11.92	66	32.0	14.6	6.49	4.09	2.40	1.70/1
	4	2	75.0	10.90	61	36.6	11.3	4.83	3.36	1.47	2.29/1
	4	3	86.0	10.10	75	32.0	15.1	5.15	3.64	1.51	2.41/1
7	7	1	73.0	11.92	70	30.4	14.4	6.67	4.28	2.39	1.79/1
	7	2	78.5	11.10	118	29.8	15.1	5.62	3.47	2.15	1.61/1
	7	3	92.0	11.10	106	34.5	17.0	5.67	.....	.....	.....
Approximate normal for cattle (2)			40–60	9–12	50–230	20–40	6–27	4–8.5	7.2	.....	.....

* 1—Oct. 30, 1942.
2—Dec. 12, 1942.
3—Feb. 20, 1943.
† 5,000–12,000 (12).

bean-meal-fed animal were associated with the diffuse cirrhotic liver found in the animal when slaughtered. The adult neutrophiles were much the same in all cases except in the last blood samples from the soybean-fed animal.

The lymphocytes were much the same in all animals.

The glucose values were similar in all animals and while slightly above that listed as normal for the adult they must be considered normal for animals of this age. Feed was never withheld before taking the blood samples, for Hodgson (*loc. cit.*) shows that this has no effect on the blood sugar of the bovine. Carbohydrate metabolism appeared to be functioning normally.

The calcium content of the blood fell between the normal ranges in all samplings and was remarkably constant. All the cholesterol values but one fell within what is listed as normal for cattle.

The non-protein and urea nitrogen of the blood was low during the period the animals were on the protein-deficient diet. The third sampling, which was taken after these animals were placed on diets containing more nitrogen, showed an increase in non-protein nitrogen and urea. All animals were metabolizing nitrogen in a normal manner. It is interesting to note that in case of the animals on ration 3 and 4 the urea values were less than one-third of the non-protein nitrogen values while the urea values for the blood of the animals on ration 5, 6 and 7 are almost half those of the non-protein values. The non-protein values are much the same whether the ration was 12.4 or 17.02 per cent protein while the urea values were about 1.5 times larger for the rations having the higher protein values.

Total serum protein was normal and much the same in all samplings. The serum albumin and globulin values for the animals on the ammoniated pulp diets were similar to those of the control animal and confirm the work of Miller (12) that serum protein is lower in young animals than in adults. The albumin-globulin ratios were similar in all cases.

The animals were slaughtered at Swift and Company under federal supervision. All animals and internal organs were pronounced normal by the federal inspectors except the liver in animal No. 7. This liver showed a diffuse cirrhosis. Macroscopic examinations of one kidney from each animal showed each to be a normal healthy kidney.

The carcasses were held in the coolers for 48 hours after the animals were slaughtered. A rib steak was then taken from each animal and tested for color and flavor at the Swift and Company Laboratories. The meat from each animal was found to be normal in color and flavor. Section of the ribs were taken from each animal and roasted and found to be normal in flavor, color and odor.

Table 4 shows the weight of the animals before they went into the coolers and the dressing percentage. The animals were not in a good grade of finish for the experiment was not designed for this purpose. However, a very small amount of fat was deposited on the inside of the ribs and they brought

TABLE 4

The weight of the animals when dressed and before going into the coolers

Animal No.	Dressed wt. before going into cooler	Dressing percentage*
	lbs.	
3	300	52.00
4	299	51.73
5	278	50.27
6	254	51.94
7	339	55.21

* Based on feed lot wt.

$10.25 per hundred weight. The soybean animal had a slightly higher dressing percentage than the animal fed ammoniated pulp.

Part of the liver, one kidney and five ribs were taken from each carcass and analyzed for the constituents shown in table 5. Since the cuts from the different steers contained different amounts of fat some variation in the analyses from animal to animal is expected.

The results on the ribs, livers and kidneys show that the animals fed ammoniated pulp contained much the same amount of fat, total protein, water-soluble protein, coagulable protein and moisture as the soybean-fed animal. This fact, together with the growth increments, is conclusive evidence that the animals were using the ammonia added to the dried beet pulp for their protein metabolism.

TABLE 5

Composition of tissue (moisture free basis)

Animal number	Fat	Total protein	H_2O sol. protein	Coagulable protein	Moisture
	Rib cut				
	%	%	%	%	%
3	31.7	65.3	14.8	7.1	70.3
4	19.5	73.4	16.2	9.3	72.2
5	23.3	74.9	17.0	7.8	72.9
6	13.9	83.1	19.7	9.6	75.1
7	21.3	75.1	17.5	9.0	73.1
	Livers				
3	7.1	69.0	27.8	12.0	71.6
4	5.6	72.0	26.2	11.5	71.4
5	4.1	67.8	30.0	15.2	71.0
6	5.6	67.8	26.5	11.3	71.7
7	6.7	73.1	26.2	15.0	69.9
	Kidneys				
3	30.3	60.3	29.0	9.5	73.8
4	19.7	70.3	32.6	14.6	76.1
5	35.1	58.7	25.6	9.2	70.8
6	32.7	56.5	28.3	7.9	73.5
7	38.7	49.5	26.0	6.9	71.1

DISCUSSION OF RESULTS

Animals on the basal diet grew very little while those on the basal ration plus ammoniated pulp for 225 days grew as much as 81.34 per cent of the growth promoted by the toasted soybean meal. Animals on a low protein diet and then changed to a diet in which the nitrogen was raised only by the ammoniated pulp grew 102 and 107 per cent of that of the soybean meal animal. Ration 6 gave only about half as good results as soybean meal with one animal but gave about 84 per cent of soybean meal when fed to another animal. The animal which did so poorly on ration 6 grew 95.55 per cent as well as the soybean meal animal when placed on ration 4. The animals on the ammoniated diet grew at the rate of 1.5, 1.65, 1.59 and 1.87 pounds per day while those animals changed from the basal ration to a ration containing ammoniated pulp grew at the rate of 2.0 pounds per day. The

TABLE 6

Basal rations of three non-protein nitrogen experiments for growing Holstein calves

	After Hart *et al.* (3)		Present experiment
	Holstein males	Holstein females	Holstein males
	lbs.	*lbs.*	*lbs.*
Yellow corn	29.5	20.0	.....
Dried beet pulp (plain)	.....	.....	60.0
Ground timothy	47.0	47.5	16.0
Starch	20.0	24.0	20.79
Corn molasses	.....	10.0	.....
Steamed bone meal	2.0	2.0	2.0
Salt	1.0	1.0	1.0
Cod liver oil	0.5	0.5	0.21
Total	100.0	105.0	100.0

soybean-meal-fed animal grew at the rate of 1.96 pounds per day. It is interesting to compare these results with those of the two experiments reported by Hart *et al.*, since Holstein calves were used in all three experiments and the diets were very similar except that sugar beet pulp was used in place of yellow corn and the amount of timothy varied somewhat. This comparison is shown in tables 6 and 7.

The animals on urea and ammonium bicarbonate grew at the rate of 0.8 to 1 pound per day while those on ammoniated pulp for 225 days grew at the rate of 1.53 to 1.64 pounds per day.

It has been shown (13) that ruminants are able to use non-protein nitrogen compounds because the microflora in their stomachs convert such nitrogen into bacterial protoplasm protein.

The enzymes pepsin, trypsin and peptidases then act on the proteins to liberate amino acids in the intestines where they are absorbed into the blood stream.

TABLE 7

Growth rates of Holsteins on certain nitrogenous rations

Holstein male calves[a] (Hart *et al.*)				Holstein female calves[b] (Hart *et al.*)				Holstein male calves (present experiment)[c]			
Ration			Daily growth (290 days)	Ration			Daily growth (365 days)	Ration			Daily growth (225 days)
No.	N supplement	% protein (N × 6.25)		Basal	N supplement	% protein (N × 6.25)		Basal	N supplement	% protein (N × 6.25)	
			lbs.				*lbs.*				*lbs.*
1—Basal	.....	6.00	0.693	Basal	.....	5.38	0.0	Basal	.....	6.94	0.279
				"	1.4 lbs. Urea	9.49	1.0	"	A.P.*	17.02	2.000[d]
2— "	Urea	18.00	1.000	"	1.4 lbs. Urea	9.49	0.80	"	" "	6.94	0.203
				"	4.7 lbs. Casein	.....	1.50	"	" "	17.02[d]	2.091[d]
3— "	NH_4HCO_3	18.00	0.951	"	2.8 lbs. Urea	13.70	0.90	"	" "	12.42	1.527
4— "	Casein	18.00	1.472	"	4.3 lbs. Urea	17.64	0.90	"	" "	12.42	1.618
5— "	.....	.....	.....	"	11.1 lbs. NH_4HCO_3	17.04	0.90	"	" "	17.02	1.591
6— "	.....	.....	.....	"	11.0 lbs. Casein	15.52	1.2	"	" "	17.02	0.976
								"	" "	12.42	1.869
7— "	.....	.....	.....	.....	.....	.....	.....	"	Toasted soybean meal	16.42	1.956

[a] Animals were 275–375 pounds at beginning of experiment and 575–700 pounds at end of experiment.
[b] " " " 250–290 " " " " " " " 592–678 " " " " " "
[c] " " " 174–335 " " " " " " " 489–614 " " " " " "
[d] Rations were changed during the experiment.
* Ammoniated pulp.

Rose *et al.* (15) have shown that the amino acids lysine, tryptophane, histidine, phenylalanine, leucine, isoleucine, threonine, methionine, valine and arginine are indispensable for the rat and dog. Since the animals on ammoniated pulp did not grow as well as the one on soybean meal it would appear that either all of the essential amino acids were not synthesized or not synthesized in sufficient quantity to give maximum growth. This leads to the interesting question of whether the growth promoting ability of ammoniated pulp for ruminants would be increased when fed in practical diets containing normal and multiple supplementary nitrogen sources such as barley, corn and alfalfa hay with these materials supplying less natural protein than is needed by the animal for maximum growth. Under such conditions if the growth were not maximum the question of supplying in an economical form those essential amino acids not synthesized by the ruminants, should be an interesting and fruitful study.

SUMMARY

1. An experiment on the ability of Holstein calves to grow on ammoniated plain sugar beet pulp has been conducted.

2. The experiments indicate that the animals can use such nitrogen sources for their nutritional needs. They grew at the rate of about 1.6 pounds per day as compared with 1.96 pounds for one animal on toasted soybean meal.

3. Animals fed a diet in which starch was substituted for molasses grew as well as those on rations containing the molasses. This indicated that the soluble carbohydrate did not further the use of the nitrogen by the microorganisms in the digestive tract any better than starch.

4. The animals grew just as well on rations 12.42 per cent protein as those on 17.02 per cent protein ($N \times 6.25$).

5. No diuresis of any of the animals occurred.

6. The blood was analyzed for fifteen different constituents three times during the experiment and found to be normal.

7. The animals and their internal organs passed federal inspection at the Swift and Company yards. The soybean-meal animal had a diffuse cirrhotic liver. This is not evidence that the liver condition was connected with the soybean meal. Macroscopic examinations of the kidneys showed them to be normal.

8. Rib and liver cuts as well as one kidney from each animal were analyzed and found to be normal in protein. The color and flavor of the meat was normal.

9. Further work is indicated.

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ANNOUNCEMENT

THIRTY-NINTH ANNUAL MEETING

JUNE 20–22, 1944

CALL FOR PAPERS

May we ask all members of the association who contemplate presenting papers for the consideration of the program committee to send the titles to the chairman, Professor H. P. Davis, Dairy Husbandry Department, University of Nebraska, Lincoln 1, Nebraska, at once. Titles will be accepted until April 1, but it is desired that they be received as early as possible because the program committee cannot meet and will have to carry on its work by correspondence. Unless otherwise arranged for by the committee, all papers are not to exceed 12 minutes in length and abstracts must be in the hands of the committee chairman not later than June 1.

VOLUME XXVII MARCH, 1944 NUMBER 3

JOURNAL OF DAIRY SCIENCE

Published by the
AMERICAN DAIRY SCIENCE ASSOCIATION
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Ohio State University, Columbus, Ohio

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ABSTRACTS OF LITERATURE

BACTERIOLOGY

58. Significance of Adequate Controls in Absolute Sterility Determinations. D. C. Foord, C. L. Crane, and B. S. Clark, Res. Dept., Amer. Can Co., San Francisco, Calif. Food Res., *8*, No. 6: 489. Nov.–Dec., 1943.

Data accumulated in routine bacteriological examination of paper milk containers over a period of three and one-half years (30,718 tests) by the rinse test of the A.P.H.A. Standard Methods, indicated 78.5% were sterile. If a correction for technique error is made, 97% of the containers were sterile. The non-sterile containers showed fewer than three bacteria per container or approximately 0.003 of the limit permitted by the U. S. Public Health Service Milk Ordinance and Code.

Data are included which demonstrate that the method of incubating nutrient broth in the container, as suggested in Standard Methods, yields an inaccurate measure of the organisms which the container would contribute to milk placed therein, because the container is subjected to conditions which it is not required to meet in commercial use.

Control data for the rinse test indicate that in testing for absolute sterility of any type of containers no less than one control should be made for each three containers tested. F.J.D.

59. Extracts from Irish Moss as a Substitute for Agar in Bacteriological Culture Media. A. W. Walker and A. A. Day, Dept. of Bact., Northwestern Univ., Medical School, Chicago. Food Res., *8*, No. 6: 435. Nov.–Dec., 1943.

The possibility of using the gelatinous extract of *Chondrus crispus* (Irish Moss) as a substitute for agar, which is becoming a "critical material," was investigated. The particular product used was "carrageen" made by the Krim-Ko Co. of Chicago, Illinois, to which the manufacturers have given the name "Carragar" when used as a substitute for agar.

Results indicated that "Carragar" can be used as a solidifying agent in bacteriological culture media. The gel is not as firm as agar, the melting point is lower and it is more easily hydrolyzed. However, it is satisfactory for most purposes and can be made firmer by additions of agar to it. Neutralization of the "Carragar" before heating prevents its hydrolyzation.

"Carragar" produced comparable results, in all of the media employed in the study, to those obtained with agar. F.J.D.

60. A Comparative Study of One Per Cent and Five Per Cent Solutions of 30 to 40 Mesh Gelatins for Bacteriological Examination. TECH. COM. OF THE EDIBLE GELATIN MFGRS., Res. Soc. of America. Food Res., *8*, No. 6: 429. Nov.–Dec., 1943.

One per cent solutions of 30- to 40-mesh gelatin are preferable to five per cent solutions for bacteriological examination, in the judgment of the committee. While no results were obtained with coarsely ground or flake gelatins, it is suggested that five or ten per cent solutions will probably yield more reliable results. F.J.D.

61. Nutritional Studies on Streptococcus Lactis. I. An Unidentified Growth Factor Found in Yeast Extract. F. R. SMITH, Univ. of Calif., Davis. Jour. Bact., *46*, No. 4: 369. Oct., 1943.

The author describes the preparation and some characteristics of an unknown substance in yeast extract that is essential for the growth of certain strains of *Streptococcus lactis*. This growth factor is apparently not a known vitamin and could not be replaced by a combination of amino acids. D.P.G.

62. Heat Resistant Organisms in Milk Supplies. W. D. DOTTERER, Dir. of Labs., Bowman Dairy Co., Chicago, Ill. Jour. Milk Technol., *6*, No. 5: 269. Sept.–Oct., 1943.

It is quite common to find a great many milk supplies containing thermoduric bacteria. Some areas are a greater source of trouble from these organisms than others.

Utensils have generally been the source, with milking machines involved more frequently than others; however, the cow's udder may also be the source.

High-temperature short-time pasteurization will generally give higher counts than the vat system of pasteurization.

Great care in the cleansing and sterilizing of utensils is necessary for the elimination of thermoduric organisms from milk supplies. L.H.B.

BREEDING

63. Inhibition of Sperm Glycolysis and Reversibility of the Effects of Metabolic Inhibitors. HENRY A. LARDY AND PAUL H. PHILLIPS, Dept. Biochem., Univ. Wis. Jour. Biol. Chem., *148*, No. 2: 343. 1943.

Using a 0.02 M glucose solution as a substrate for washed bull spermatazoa the following inhibited glycolysis and their effect was reversible: cyanide, quinone, fluoride and azide; while maleate, hydroquinone, iodo-

acetate and glyceraldehyde inhibited glycolysis but their effect was not reversible.

"In yolk-buffer some specimens of bull spermatazoa could be almost completely inactivated by fluoride for several days and upon transfer to fresh yolk-buffer vigorous motility was regained." A.O.C.

64. Inhibition of Sperm Respiration and Reversibility of the Effects of Metabolic Inhibitors. HENRY A. LARDY AND PAUL H. PHILLIPS, Dept. Biochem., Univ. Wis. Jour. Biol. Chem., *148*, No. 2: 333. 1943.

The effect of 17 different metabolic inhibitors on the respiration of bull spermatazoa and the reversibility of the effect of these inhibitors is given. Cyanide, malonate and benzoate inhibit the respiration but not the glycolysis of the sperm and are toxic to motility only when glucose is absent. This is further evidence of the ability of 2 separate metabolic processes, *i.e.*, oxidative and glycolytic, to furnish energy for motility of bull sperm. A.O.C.

BUTTER

65. There Will Always Be Quality. R. E. ELDRED, The Great Atlantic & Pacific Tea Co., Chicago. Natl. Butter and Cheese Jour., *35*, No. 1: 8. Jan., 1944.

The customer today wants quantity rather than quality. Inferior cream and butter command the same prices as better products. Some factors causing quality deterioration are: delays in cream pick-ups, farm labor shortage, relative prices for farm and dairy products, curtailment of educational field work, discrepancies in official gradings, lack of food regulatory personnel, increased production of unsalted butter and differences of point values in farm and creamery butter. But quality has not been forgotten because critical customers are asking for better butter and the army, which is buying good table butter, is educating the tastes of more men. As normal trading conditions are resumed receivers will be more strict, the Food and Drug departments will again do routine checking and the natural pride of butter makers will all tend to improve quality. Large milk drying operations will produce fine cream, some of which will be used for butter, and small operators may have to develop cooperative field services to compete with these larger concentrators. W.V.P.

66. Looking into the Future of Creamery Operations. H. E. BEHLMER, Cherry-Burrell Corp., Chicago. Natl. Butter and Cheese Jour., *34*, No. 12: 18. Dec., 1943; and *35*, No. 1: 36. Jan., 1944.

Future efforts to improve quality and lower manufacturing costs will probably require changes in methods and machinery. Cream quality will

be improved by education of producers, development of better-equipped cream receiving units, and more efficient utilization of milk-solids-not-fat. Innovations in creameries will include: continuous butter manufacture without churns, more general use of the vacuum-type pasteurizer, better cooling practices, and coolers designed to control temperatures and deliver cream to churns instead of holding tanks. Other significant changes expected are: greater use of stainless steel in vats, pipes, pumps and refrigerating units, better sanitation, more general use of storage tanks instead of coil vats and the use of better lubricants. W.V.P.

67. Determination and Content of Carotene and Vitamin A in Wisconsin Butter. S. BERL AND W. H. PETERSEN, Dept. Biochem., Univ. Wis., Madison. Jour. Nutr., *26*, No. 5: 527–538. Nov., 1943.

A solvent extraction is described for the determination of carotene and vitamin A in butters, utilizing the yellow color of the carotene for its determination and the Carr-Price reaction for the determination of vitamin A.

Diacetone alcohol, 94%, proved to be superior to 92% methanol for the extraction of non-carotene pigments.

Twenty-two samples of butter made during the week of March 23, 1942, by creameries in southwestern Wisconsin, and 20 samples of butter from the same creameries in July, 1942; and samples of butter made in September and January collected from various sections of Wisconsin were used in this study.

The March butters averaged 9,500 I.U.; the July and September butters 18,000 I.U.; and the January butters 10,500 I.U. per pound. In the summer butters about 75% of the total natural butter pigment was found to be carotene, in the winter butters 60 to 65% of pigment was carotene.

Storage of butter at -22 to $-23°$ F. as long as 8 months did not result in a loss of carotene or of vitamin A. C.F.H.

68. The Iron Content of Butter and Its Relation to the Butter Wash Water. J. B. LINNEBOE, Alberta Dept. Agr., Edmonton. Sci. Agr., *24*, No. 2: 64. 1943.

Samples of butter from 146 churnings representing 27 creameries were analyzed for iron. The iron content of the wash water from 26 of these creameries were also determined.

The iron content of the butter ranged from 0.4 ppm. to 3.3 ppm. The butter possessing the lower iron content tended to grade higher and there was also a tendency towards uniformity in the iron content of the butter from the same creamery, particularly the butter of first grade.

The iron content of the wash water was shown to affect the iron content of the butter. Where portions of churnings were removed from the churn and washed with distilled water or natural water, low in iron, all the first grade butter contained less than 1.0 ppm. of iron. O.R.I.

CHEESE

69. Cottage Cheese from Soybean Curd? ANONYMOUS. Milk Dealer, *33*, No. 3: 27–56. Dec., 1943.

Following a description of making cottage cheese from soybeans' milk, as released by Science Service, a survey of the possibilities of making a substitute cottage cheese by dairy plants is made. Methods of treating soybean milk to produce a curd comparable in many respects to regular cottage cheese made from skimmilk have been to culture milk with lacto-bacillus or soy acidophilus organism; to use an alkali (magnesium chloride) to coagulate the soybean milk or to curdle the milk with a weak acid. A patent for manufacturing has been applied for by one dairy plant. The possibility of providing a substitute cottage cheese under existing shortages of skimmilk, which possesses some of the texture, flavor, acidity and moisture content characteristics of regular cottage cheese is discussed. C.S.T.

70. Starters for Cheese Making. G. M. MOIR, Dairy Chemist. New Zeal. Dept. Agr. Bul. No. 162.

This bulletin brings together the more important research and practical observations on the making of starters as applied to cheese factory conditions in New Zealand.

Starters are used to ripen the milk before renneting, to secure proper acidity during the making process, to restrain undesirable bacteria, and to aid in cheese ripening. The starter must contain the right bacteria, and no others, in active condition capable of developing acidity at the proper rate. The cultures in New Zealand are often pure strains of *Str. cremoris*, but usually *Str. lactis* and *Str. citrovorus* are also present.

Bacteriophage is a common cause of limited acid development. The phage infection usually occurs from the "whey frog" from a whey separator, from dust, or from contamination from phage-infected starters. Some other conditions that cause slow acid development are low temperatures of incubation, growth-retarding substances in the milk, aeration of the milk after pasteurization, and too small inoculation, but phage contamination is most important.

The objection to excessive acidity (1.0–1.2%) in former years was due to poor starters. Actually 0.65–0.85% is quite satisfactory and overripening is no longer so common due to use of proper bacteria.

Special milk should be selected for starters. Special starter rooms in cheese factories are desirable. Several starters are preferred to one to assure against complete failure. Mother cultures are best carried in glass containers. Stress is given to cotton plugging the openings for inoculation for the main and mother starter and of flaming these openings when transfers are made to avoid phage contamination. Details of making the starters are given and the Whitehead and Cox vitality test is described.

It is pointed out that phages have not been troublesome in America and that mixed cultures are probably preferable to single strains to control phage difficulties. A.C.D.

CHEMISTRY

71. **Modifications of the Swift Stability Test.** R. W. RIEMENSCHNEIDER, J. TURER, AND R. M. SPECK, Eastern Regional Res. Lab., U.S.D.A., Philadelphia, Pa. Oil and Soap, *20*, No. 9: 169–171. Sept., 1943.

An all-glass aeration tube for use in the accelerated method of determining stability of fats was found to have several advantages over the rubber-stoppered test tubes. An improved air-distributing apparatus is also described. The capacity of the apparatus has been increased three-fold by the adoption of a procedure which permitted the use of only one tube for each test sample.

Factors influencing the peroxide values as determined by adaptation of Wheeler's method were investigated. J.L.H.

72. **The Development of a Practical Antioxidant for Lard and Shortening.** A. LIPS AND W. D. MCFARLANE, Dept. of Chem., Macdonald College (McGill Univ.), Ste. Anne de Bellevue, Quebec, Canada. Oil and Soap, *20*, No. 10: 193–196. Oct., 1943.

Wheat germ oil, extracted by means of ethylene dichloride and fortified with citric acid, was found to be an efficient antioxidant in lard and shortening. The wheat germ oil supplied the phenolic type of antioxidant in the form of tocopherols and the citric acid, the acidic component necessary in an antioxidant intended for use in both animal and vegetable fats and oils.

J.L.H.

73. **The Solubility of Gases in Butter Oil, Cottonseed Oil, and Lard.** P. S. SCHAFFER AND H. S. HALLER, Bur. of Dairy Indus., U. S. D. A., Washington, D. C. Oil and Soap, *20*, No. 8: 161–162. Aug., 1943.

The solubility of hydrogen, oxygen, air, nitrogen, and carbon dioxide at 40° C. is slightly higher in butter oil than in cottonseed oil and lard. The solubilities of the above gases in butter oil at 40° C. and 60° C. are shown in the following table:

Gas	Gas dissolved in 100 ml. butter oil	
	40° C.	60° C.
	ml.	*ml.*
Oxygen	14.2	12.7
Nitrogen	8.9	7.9
Hydrogen	5.4	6.8
Air	10.1	9.6
Carbon dioxide	109.5	91.0

J.L.H.

74. The Application of the Ferric Thiocyanate Method to the Determination of Incipient Rancidity in Fats and Oils. A. LIPS, R. A. CHAPMAN, AND W. D. MCFARLANE, Dept. of Chem., Macdonald College (McGill Univ.), Ste. Anne de Bellevue, Quebec, Canada. Oil and Soap, *20*, No. 11: 240–241. Nov., 1943.

A colorimetric method for determining fat-peroxides in whole milk powder, previously reported by the authors, has been modified for use in fats and oils. The method is based on the oxidation of ferrous to ferric iron by the peroxides present in oxidized fats. The ferric iron is determined as ferric thiocyanate; the intensity of the color being measured with a Coleman spectrophotometer set at 485 mμ. J.L.H.

75. A Rapid Test for Alpha Dicarbonyls. L. O'DANIEL AND L. B. PARSONS, Res. Dept., Lever Bros. Co., Cambridge, Mass. Oil and Soap, *20*, No. 4: 72–74. Apr., 1943.

When alcoholic caustic potash is added to auto-oxidized fats and oils more or less highly-colored solutions are formed, the color depending upon the type of fat and the extent of oxidation. The color is probably due to quinoid compounds formed by aldol condensation of alpha-diketones in a manner analogous to the formation of para-xyloquinone from diacetyl. Linoleic and possibly linolenic acids or esters are undoubtedly the source of the quinoid compounds. A rapid test for alpha dicarbonyls in fats and oils is described. J.L.H.

76. The Antioxidative Behavior of Vegetable and Animal Fats. CALVIN GOLUMBIC, Bio-Chem. Lab., State Univ. of Iowa, Iowa City. Oil and Soap, *20*, No. 6: 105–107. June, 1943.

Autoxidizing animal fats exhibit a well-defined induction period, the end of which coincides with the development of oxidative rancidity. Vegetable fats, however, do not have a sharply defined induction period and they show oxidative rancidity before the period of relatively rapid oxygen uptake and peroxide formation.

A kinetic study of the oxidation of tocopherol during the induction periods of animal and vegetable fats yielded information on possible causes of the differences in the induction periods. The oxidation of tocopherol in both fats is accompanied, in the early stages, by the formation of tocoquinones. In animal fats the complete disappearance of added tocopherol marks the end of the induction period and the beginning of organoleptic oxidative rancidity.

Chroman, -5, 6-quinones appear during the course of the induction period of autoxidizing vegetable, but not animal fats. These O-quinones, derived from unknown precursors, are antioxidants and their relatively slow oxidation rate as compared with tocopherol permit them to act after the

disappearance of tocopherol. This action offers an explanation for the absence of sharp induction periods of vegetable fats.

Tocopherols are decreasingly effective as antioxidants when employed at higher levels. This accounts for the previously recognized ineffectiveness of tocopherols and inhibitol concentrates when added to vegetable fats.

J.L.H.

77. Estimation of Vitamin A in Food Products. BERNARD L. OSER, DANIEL MELNICK, AND MORTON PADER, Food Res. Labs., Inc., Long Island City, N. Y. Jour. Indus. and Engin. Chem.. Analyt. Ed., *15*, No. 12: 724. Dec., 1943.

Modification of the antimony trichloride method for determination of vitamin A in food products, including dairy products, was made. Corrections are allowed for the presence of inhibitors of the color development, for temperature effects, for variations in the reagent, for turbidities produced in the course of the color development, and for extraneous color present in the final test solution. The unreliability of the direct spectrophotometric method for the assay of foods is demonstrated. The reaction of carotene with antimony trichloride was studied. B.H.W.

78. Factors Affecting the Stability of Cottonseed Oil. A Study of the Antioxygenic Activity of Alpha-Tocopherol. C. E. SWIFT, W. G. ROSE, AND G. S. JAMIESON, Bur. of Agr. Chem. and Engin., U.S.D.A., Washington, D. C. Oil and Soap, *19*, No. 10: 176. 1942.

The results secured suggest that the antioxygenic activity of the tocopherols is due to their reactivity towards the active peroxides. The rate and extent of peroxide accumulation during the induction period was found to be dependent on the tocopherol content of the oil. The tocopherols function most effectively at lower levels of concentration and with decreasing efficiency at higher levels. Small amounts of the cephalin fraction markedly retarded the rapid initial rate of oxidation of α-tocopherols. This effect demonstrates the synergism of an "acid-type" substance acting with a "phenolic-type" antioxidant. J.L.H.

79. The Oven and Aeration Methods as Means of Accelerating Fat Oxidation. F. C. EWBANK AND I. A. GOULD, Dept. of Dairying, Mich. State Col., East Lansing, Mich. Oil and Soap, *19*, No. 12: 205. 1942.

A comparison was made of the aeration and hot oven methods of accelerating butter oil oxidation at 100° C. The rate of peroxide formation was used in determining the induction period; a peroxide value of 5 being arbitrarily chosen as the end of the induction period. The two methods were

found to be equally reliable when careful technique was employed and where the influence of certain variable factors were controlled. With the oven method especial attention must be given to temperature control, air agitation and arrangement of the samples in the oven if uniform results are to be obtained. The oven method gave a shorter induction period than the aeration method but the extent of oxidation over a relatively long period was less. J.L.H.

80. The Antioxygenic Action of Phosphoric Acid in Association with Tocopherols and Hydroquinones. CALVIN GOLUMBIC, Bio-Chem. Lab., State Univ. of Iowa, Iowa City, Ia. Oil and Soap, *19*, No. 10: 181. 1942.

Phosphoric acid was found to retard the oxidation of tocopherol in autoxidizing fats and thus to increase its antioxygenic activity. The stabilizing capacity of hydroquinones was likewise found to be increased by small amounts of phosphoric acid. The data secured suggest that this synergistic action is due to the shifting to the left, of the hydroquinone $\rightleftarrows$ quinone equilibrium. The tocohydroquinone $\rightleftarrows$ tocoquinone equilibrium is a special case of this relationship in which phosphoric acid also catalyzes the cyclization of tocohydroquinone to tocopherol, thus regenerating the antioxidant. J.L.H.

81. Vitamin A Added to Fats as Related to Stability During Baking. E. E. RICE, H. C. BLACK, G. T. CARLIN, AND H. E. ROBINSON. Swift & Co., Chicago, Ill. Oil and Soap, *19*, No. 9: 164. 1942.

The Carr-Price color reaction and U.S.P. bio-assays were used to determine the stability of vitamin A in various types of baked goods prepared with fats fortified with vitamin A. In bread, biscuits and cake which are relatively low in fat and baked under moderate conditions it appears that 80 to 100% of the vitamin survives the baking process. When the fat content is higher and the baking conditions more severe as in pie crust, considerable vitamin A destruction is likely to occur depending on the extent of the baking. J.L.H.

CONCENTRATED AND DRY MILK; BY-PRODUCTS

82. The Nitrogen Distribution in Dried Milk. U. S. ASHWORTH AND HARRIS O. VANORDEN, Div. of Dairy Hus., Agr. Expt. Sta., Pullman, Wash. Jour. Milk Technol., *6*, No. 5: 272. Sept.–Oct., 1943.

Samples of spray-dried skimmilk were analyzed to determine whether the nitrogen distribution differed from that of normal skimmilk.

The results obtained when calculated to a moisture-free basis were found to agree very well with those reported in the literature for fresh skimmilk

with the exception of the albumin-plus-globulin fraction. Out of 32 samples tested only four contained measurable amounts of this fraction. The amount found, no doubt, was associated with the heat treatment that the samples received prior to drying. L.H.B.

83. A Laboratory Spray Drier. A. H. Woodcock and H. Tessier, Natl. Res. Labs., Ottawa. Canad. Jour. Res., *A, 21,* No. 9: 75. 1943.

A laboratory model of a cyclone-type spray drier which in operation has produced dried milk and dried egg of good commercial quality is described. The main drying chamber is an inverted cone 2 feet in diameter and 5 ft. 2 in. in height made of galvanized metal and covered with insulating materials. Adjustments are provided for inlet air temperature, total air flow, angular velocity of air in the drying chamber, quantity of liquid introduced, and spraying technique. The air is supplied by a fan similar to the type used in a large household vacuum cleaner and is heated by a thermostatically controlled, 5000-watt electric element. The maximum capacity is about four liters of liquid per hour. O.R.I.

DISEASE

84. Newer Methods for the Control of Bovine Mastitis. G. R. Spencer, Dept. of Vet. Sci., Univ. Wis. Milk Dealer, *33,* No. 1: 52. Oct., 1943.

To control the spread of mastitis by the dairyman, the infected animals must be "spotted" "by the strip cup and the Hotis test." Both tests are needed to determine both new or "carrier" cows. Segregation is recommended in lieu of slaughter at the present time as a means of preventing spread of infection. Segregated animals should be milked and handled by special personnel if possible, and if not, every sanitary precaution exercised to prevent spread of infection such as reduced feeding to facilitate treatment; thorough milking of all quarters; avoidance of teat injuries and specific medicinal treatment to cure and reduce extent of infection, are recommended as control measures. C.S.T.

85. Production and Prevention of Bloat in Cattle on Alfalfa Pasture. H. H. Cole, S. W. Mead, and W. M. Regan, Univ. Calif., Davis, Calif. Jour. Anim. Sci., *2,* No. 4: 285–294. Nov., 1943.

Bloat was produced at will in dairy cows by depriving them of hay and bedding for at least 48 hours and pastured on thick stands of immature alfalfa that was 8 to 14 inches high. A thick stand makes possible rapid ingestion. It was necessary to use pastures free from weeds and contaminating grasses. Cows tend to select considerable amounts of coarse, seemingly unpalatable weeds where succulent alfalfa is abundant.

The availability of the water in the pasture did not affect the incidence of bloat. The feeding of alfalfa hay reduced the incidence and severity of bloat but was not completely effective. Bloat was effectively controlled on alfalfa pasture by feeding Sudan hay in the corral or in the pasture. Also, pasturing on Sudan at night before placing the cows on alfalfa pasture appeared to be an effective means of controlling bloat. Cows ruminated only one-half as much on alfalfa pasture as on Sudan pasture. Apparently both rumination and belching are readily induced by Sudan because of scabrous leaves. C.F.H.

86. A Cheese-Borne Epidemic of Typhoid Fever. JACQUES GAUTHIER AND A. R. FOLEY, Min. of Health and Soc. Welfare, Quebec. Canad. Jour. Pub. Health, *34*, No. 12: 543. 1943.

An outbreak of typhoid fever which resulted in 40 cases and six deaths is described. Evidence indicated that milk supplied to the cheese factory became contaminated from a carrier working on a farm. The cheese was of the Cheddar type and was consumed when about ten days old. Butter made in the same factory from pasteurized cream was not incriminated. In addition to the control measures taken the following recommendations are made:

1. Early notification of each case of enteric fever.
2. General check-up of typhoid carriers.
3. Pasteurization of the cheese milk or at least the holding of the product for a three-month period before consumption. O.R.I.

87. Effects of Disease on Nutrition. I. Absorption, Storage, and Utilization of Vitamin A in the Presence of Disease. SAMUEL SPECTOR, CHARLES F. MCKHANN, AND EMILY R. MESERVE, Dept. of Ped. and Communicable Dis., Univ. of Mich. Med. School, Ann Arbor, Mich. Amer. Jour. Dis. Children, *66*, No. 4: 376–395. Oct., 1943.

This paper is a review of the effects of disease on absorption and utilization of vitamin A. Most of the data cited were obtained with human subjects, and the review has been made from the viewpoint of human medicine. The effects of several pathological conditions are discussed, which includes jaundice, celiac syndrome, cystic fibrosis of the pancreas, infection, cirrhosis of the liver, valvular lesions of the heart, malignant neoplastic tissue, disorders of the thyroid, and allergy.

It is pointed out that malnutrition may be the result of organic disturbances within the patient. For example, during infections there develops rather regularly a decrease in the ability of the intestinal tract to absorb carotene and vitamin A. This decreased absorptive power occurs even with infections that do not primarily involve the gastrointestinal tract.

It is not possible to make a complete review of this article, as it in itself is a review. Sixty-two references are given. R.K.W.

FEEDS AND FEEDING

88. Feeding Standard Equation for Cows and Goats in Milk. W. L. GAINS, Univ. Ill., Urbana, Ill. Jour. Anim. Sci., *2*, No. 4: 304–313. Nov., 1943.

The precision data used in this investigation were reported in the literature from the Pennsylvania Agricultural Experiment Station. One hundred seven observations on 10 cows were used in the formulation of a standard equation for lactating cows. The feeding standard equation for the milking cow is as follows:

DN = 0.008 W + 0.3 F.C.M., in which DN = daily digestible nutrients intake in pounds, W = live weight in pounds, F.C.M. = daily milk-energy yield in pounds 4% milk. C.F.H.

89. Studies on the Alimentary Tract of Merino Sheep in South Africa. VI. The Role of Infusoria in Ruminal Digestion with Some Remarks on Ruminal Bacteria. J. G. VAN DER WATH AND S. J. MYBURGH, Section of Biochem., Onderstepoort, Pretoria, South Africa. Onderstepoort Jour. Vet. Sci. and Anim. Indus., *17*, Nos. 1 & 2: 61–85. Oct., 1941.

A technique is described for the preservation and counting of ruminal infusoria.

The changing of the ration of sheep from alfalfa and corn to corn alone resulted in extinction of the large type of infusoria and a reduction of all types following the cessation of rumination. Appetite of the sheep was adversely affected. A ration of wheat straw alone resulted in the starvation of ruminal infusoria. The addition of corn to the wheat straw resulted in a marked increase in the number of infusoria. The feeding of green alfalfa only brought about a reduction in the number of infusoria.

Season fluctuations of ruminal infusoria of grazing sheep are described. The amount of protein in pasture had a significant influence on the density of the infusoria population.

The rate of digestion of fine yellow corn meal by starved infusoria in the rumen was studied. Yellow corn particles were found in the infusoria one minute after being placed in the rumen. Disintegration of corn particles was observed within the infusoria. The so-called glycogen granules observed within the plasma of the infusoria were shown to be glycogen-synthesizing bacteria. The food and bacteria ingested by the infusoria were digested by enzymes secreted by the bacteria present. In an experiment where the infusoria and bacteria were destroyed by feeding copper sulphate and the rumen inoculated with starch-digesting bacteria, it was shown that infusoria are not necessary for starch digestion.

Infusoria engulfed particles of cellulose. The digestion of cellulose within the body of the infusorium is primarily due to cellulose-digesting bacteria ingested by the infusorium. C.F.H.

90. Brown Silage from Atlas Sorgo—Chemical Composition and Apparent Digestibility as Determined by Feeding to Dairy Cows. H. E. BECHTEL, F. W. ATKESON, AND J. S. HUGHES, Kans. Agr. Expt. Sta., Manhattan, Kans. Jour. Anim. Sci., *2*, No. 4: 295–303. Nov., 1943.

Chemical analyses of five brown silage samples and one sample of high moisture chopped brown fodder were compared with those of normal green Atlas sorgo silage. Three silages were used in digestion trials. (1) Normal green silage from a wire and roofing paper silo in which maximum temperature during storage was 92° F. (2) Brown silage from sorgo bundle silage in which the maximum temperature during storage was 141° F. (3) Brown silage from a straw bale silo in which the maximum temperature during storage was 147.5° F.

The chemical analyses showed no appreciable difference between the brown and normal silages except for carotene and possible ash. The amount of carotene in the brown (high temperature) silages was very low.

There was a marked decrease in the coefficients of digestibility for all nutrients except ether extract in the brown silage. The degree of digestibility appeared to depend on the maximum temperature during storage. Protein was most affected, the apparent digestibility averaged 55% for normal silage, 23 and 4% for the two brown silages.

Brown silage was less palatable than normal silage. C.F.H.

91. Biological Methods of Measuring the Protein Values of Feeds. H. H. MITCHELL, Div. Anim. Nutr., Univ. Ill., Urbana, Ill. Jour. Anim. Sci., *2*, No. 4: 263–277. Nov., 1943.

The evaluation of biological methods of measuring the protein values of feeds is discussed. Conditions considered essential in measuring protein values of feeds are as follows:

1. The measurement of protein utilization by studying the nitrogen economy of the animal.

2. The measurement selected must involve the use of dietary protein in maintenance as well as in production.

3. The imposition of comparable levels of protein feeding.

4. The control of the food intake of comparable animals, which generally means the equalization of food consumption. C.F.H.

92. Feeding Dairy Stock in Wartime. H. S. WILLARD. Wyo. Agr. Expt. Sta. Bul. 263, 19 pages.

Although an experiment station publication this is primarily an exten-

sion bulletin. Information is furnished on the use and importance of hay and pasture in dairy production, correct apportionment of grain allowances under differing conditions, grain mixtures suitable for feeding with different kinds of roughage, and wartime production of dairy calves and heifers. Several tables are included for the guidance of the stockman. J.G.A.

93. The Influence of Dietary Fat of Varying Unsaturation on the Component Acids of Cow Milk Fats. T. P. HILDITCH, Dept. of Indus. Chem., Univ. of Liverpool, AND H. JASPERSON, Res. Dept., Messrs. J. Bibby and Sons, Ltd., Liverpool. Biochem. Jour., *37*, No. 2: 238–243. 1943.

In most feeding trials wherein different oils or fats have been fed to dairy cows the results have been interpreted from the over-all or gross change in the degree of saturation of the butter fat produced, but in this particular study an attempt was made to differentiate between the component fatty acids responsible for this gross change.

Fifteen cows were divided into five groups and fed (1) a basal diet; (2) basal diet plus peanut oil which is highly unsaturated, having an I.v. (iodine value or iodine number) of 88; (3) basal diet plus partially hydrogenated peanut oil having approximately the same I.v., 45, as soft butter fat; (4) basal diet plus hydrogenated peanut oil which was almost completely saturated—I.v. of 17; and (5) basal diet plus palm kernel oil which is naturally highly saturated, I.v. of 17.

Groups (2) and (3) produced butter fat with an increased amount of oleo-glycerides and a decrease in the butyric to caprylic glycerides. Group (5) produced butter fat with the lauro-glycerides three-fold greater and myristo-glycerides 20% greater than the control, but the oleo- and palmito-glycerides showed a slight decrease. Group (4) receiving the highly hydrogenated or saturated peanut oil gave butter fat more nearly like the control group in its composition. This is explained on the basis that about half of the fat fed was so completely saturated that its melting point was above the body temperature of the cow and so was not assimilated. It is interesting to note that although the fats fed to groups 4 and 5 had the same I.v. they produced butterfats of quite different detailed fatty acid compositions. Peanut oil normally contains about 20% of the unsaturated acids, linoleic, arachidic, and lignoceric acids, but these did not pass into the butter fat. It seems that the mammary gland has a selective action in the absorption of oleic (as distinct from linoleic) acids.

A good review of previous work is given. A.O.C.

94. The Utilization of Urea in the Bovine Rumen. 1. Methods of Analysis of the Rumen Ingesta and Preliminary Experiments in Vivo.

R. M. Pearson and J. A. B. Smith, Hannah Dairy Res. Inst., Kirkhill, Ayr, Scotland. Biochem. Jour., *37*, No. 1: 142–148. 1943.

"The relative merits of trichloroacetic acid, sodium tungstate with sulfuric acid, and alcohol have been compared as precipitants in the estimation of non-protein-nitrogen in rumen ingesta. A description is given of the methods finally adopted for the estimation of non-protein-nitrogen, urea and ammonia."

Samples of the rumen ingesta were taken for analysis from the rumen fistula of a steer, but the authors concluded that there were so many difficulties involved in the *in vivo* experiments, especially the fact that it was practically impossible to secure a representative sample, that "the results of *in vivo* experiments of this type cannot be regarded as supplying evidence either for or against the theory that urea is converted to protein in the rumen," and because of this, they state that *in vitro* methods would be more reliable. A.O.C.

95. The Utilization of Urea in the Bovine Rumen. 2. The Conversion of Urea to Ammonia. R. M. Pearson and J. A. B. Smith, Hannah Dairy Res. Inst., Kirkhill, Ayr, Scotland. Biochem. Jour., *37*, No. 1: 148–153. 1943.

Samples of rumen ingesta taken from the gastric fistula of a steer were used in making *in vitro* studies of its action on urea.

All of the urea which would ever be likely to be fed to a cow will be converted to ammonia within an hour.

The urease preparation derived from the rumen was found very similar to the ureases from soya or jack beans in its behavior to temperature, pH and inhibitors. Preliminary attempts to obtain enzyme preparations free from bacteria proved unsuccessful. A.O.C.

96. The Utilization of Urea in the Bovine Rumen. 3. The Synthesis and Breakdown of Protein in Rumen Ingesta. R. M. Pearson and J. A. B. Smith, Hannah Dairy Res. Inst., Kirkhill, Ayr, Scotland. Biochem. Jour., *37*, No. 1: 153–164. 1943.

The liquid rumen contents taken from a gastric fistula of a steer were studied *in vitro*. In order to avoid any appreciable change in the "microbiological picture" incubation periods of only 2 to 4 hours were used. Urea was added to the liquid before incubation. Most all of the non-protein nitrogen was made up of urea and ammonia. Synthesis of protein appeared to occur to the extent of about 9 mg. N/100 gms. rumen liquid. While the total nitrogen remained constant, there was a decrease in the non-protein nitrogen accompanied by a parallel decrease in the ammonia, suggesting that the protein synthesis was from ammonia rather than from urea. This

conversion of ammonia to protein is thought to be microbiological in nature. Accompanying the protein synthesis is a protein breakdown—and either may predominate depending upon the general conditions or the substances present. A.O.C.

97. Urea as a Partial Substitute in the Feeding of Dairy Cattle. E. C. OWEN, J. A. B. SMITH, AND N. C. WRIGHT, Hannah Dairy Res. Inst., Kirkhill, Ayr, Scotland. Biochem. Jour., *37*, No. 1: 44–53. 1943.

Results are given for feeding trials with seven lactating Ayrshire cows over a period varying from 100 to 160 days wherein a third of the nitrogen of the feed was given in the form of urea and then substituted for an equal amount of nitrogen in the form of blood meal.

"The nitrogen balance and excretion data show that, although urea was partially retained by all the animals, its retention was not complete. Compared with blood meal, amounts varying from some 12 to 47% and averaging 25% of the ingested urea apparently passed through the animal without being utilized. This apparent wastage was much reduced when urea feeding was preceded by a period in which the diet had been deficient in total nitrogen.

"The milk yields of five of the seven animals were well maintained when blood meal was replaced by urea. With four of the five cows under test a rapid and significant decrease in milk yield took place when urea was removed from the food."

Data for milk yield, body weight and nitrogen balance are given for the periods. There were no significant differences in the composition of the milk. A.O.C.

FOOD VALUE OF DAIRY PRODUCTS

98. Dairy Products in the Wartime Dietary. H. H. MITCHELL, Prof. of Anim. Nutr., Univ. of Ill., Urbana, Ill. Milk Dealer, *32*, No. 10: 28–30, 78–80. July, 1943.

Despite the fact that milk supplies a generous amount of essential nutrients, its value is in the variety of ways it may be used to serve different purposes on man's menu. The processing of milk into the wide variety of products to which it lends itself show the changes even in nutritive value to which it is adapted. As an example, Limburger cheese may increase two to three times in pantothenic acid, niacin and biotin due to the curing process. Fortified oleomargarine and butter are compared nutritionally. The author deplores the advent of the role medical men are taking in formulating nutritional policies to the exclusion of scientific research, since to the medical profession nutrition "is only one of a number of fields of interest" and therefore lead to deductions not subscribed to by trained nutri-

tionists. The fortification of bread with vitamins and the possible exclusion of milk-solids-not-fat is cited as an undesirable procedure since 6% of skim milk powder added to bread "improves the growth-promoting and bone calcifying value of the bread much more than does its enrichment with the official proportions of thiamin, niacin, and iron." Skim milk powder would improve the nutritive value of all bread. The shift to greater sale and use of whole milk from the farm as a wartime need is discussed in its relationship to supplanting skim milk for animal feeding purposes which "would raise serious obstacles in the production of pork, poultry and eggs" were it carried to an extent recommended by some economists. The efficiency of the dairy cow in converting concentrate grain into milk of greater nutritive value than the grain consumed is given as 70% as compared with the 72 to 75% efficiency of the miller converting wheat into patent flour of lower nutrient value than the wheat processed. C.S.T.

99. Dairy Research and Human Nutrition. O. E. REED, Chief of Bur. of Dairy Indus., Agr. Res. Admin., U.S.D.A., Washington, D. C. Milk Dealer, *32*, No. 12: 35, 78–88. Sept., 1943.

Research in human nutrition is responsible for increased emphasis on the nutritive quality of food supplied to both our armed forces and civilians in this war, whereas quantity of food only was stressed in the last war. Dairy and animal research since the last war has made available information on what is needed to supply human nutritional needs, as to the kind and amount of nutrients required to maintain health, and the effect of lack of certain essential nutrient substances upon physical development. Research and surveys conclusively reveal the high place of milk as prerequisite for nutrition and health in the diet. The United States produces enough milk to provide a quart of milk per day per individual, but unfortunately we do not consume that quart in its entirety but instead utilize only the cream, the butterfat or the curd to the detriment of our nutritional welfare. The author traces the historical growth of the butter and cheese industry and inventions which made possible the complete utilization of the milk as it comes from the cow. The era of vitamins starting in 1912 and 1913 as the result of research gave an impetus to use of all the milk as well as to the respective merits of different vitamins. Nutritional research on vitamin A content of butter was started in 1941 and 23 states are cooperating in this nutritional survey. The "wealth of essential nutrients below the cream line" is likewise being studied. The study of the proteins, milk sugar and inorganic salts of milk but substantiate the importance of the nutritional role such ingredients play in an adequate diet, over and above the role vitamins of milk play. The role of still other constituents of milk wherein a growth factor has been shown is being studied and "every nutritive essential that we have discovered to date has been found to exist in the milk of

the cow. . . ." "Dairy products and good nutrition are inseparable. Good nutrition is fundamental for the physical, economic and moral progress of our people." By marketing the entire output of the dairy cow the dairy industry can best contribute to that progress. C.S.T.

100. War Needs Teach Nutritional Value of Milk to British Consumers. GEORGE WALWORTH. Milk Dealer, *33*, No. 3: 32–33. Dec., 1943.

Previous to the war, British fluid milk consumption averaged only one-third pint per day per capita, and efforts to subsidize milk for school children and general use largely failed. With the advent of war, however, the need for adequate nutrition and the recognition of milk as a basic food to prevent malnutrition of mothers, children and low-income groups was recognized by the British Government, and its distribution to such groups was subsidized and rationed on a preferential basis. The postwar effect of the recognition of the valuable nutritional role of milk and dairy products to the British consuming public should result in increased sale of all such products and serve as "the finest investment a government can make for the health and welfare of its people." C.S.T.

101. The Ratio of Ascorbic Acid, Riboflavin and Thiamine in Raw and Pasteurized Milk. A. D. HOLMES, C. P. JONES, A. W. WERTZ, AND J. W. KUZMESKI, Mass. Agr. Expt. Sta., Amherst, Mass. Jour. Nutr., *26*, No. 4: 337–345. Oct., 1943.

Composite milk from a herd consisting of 18 Ayrshires, 13 Guernseys, 18 Holsteins and 11 Jerseys, collected during January, February and March was used in this study. The milk was held at 40° F. for an average of 10 hours prior to pasteurization in a stainless steel vat, pasteurized by the holding process for 30 minutes at 143–145° F.

The ascorbic acid content of the raw milk ranged from 14.0 mg. to 22.5 mg. and averaged 19.7 mg. per liter; after pasteurization, the extreme values were 7.0 mg. and 19.1 mg., with an average value of 15.9 mg. per liter. The loss of ascorbic acid during pasteurization was 18.3%.

The riboflavin content of the raw milk varied from 1.35 mg. to 1.75 mg. and averaged 1.51 mg. per liter. The corresponding riboflavin values for the pasteurized milk were 1.19, 2.06, and 1.48 mg. per liter, respectively. The loss of riboflavin during pasteurization was only 2%.

The thiamine content of the raw milk varied from 0.29 to 0.35 mg. and averaged 0.33 mg. per liter; after pasteurization, the extreme values were 0.21 and 0.34, and averaged 0.30 mg. per liter. The loss of thiamine during pasteurization was 9.1%. C.F.H.

102. The Application of Chromatography to the Study of the Carotenoids of Human and Cow's Milk. S. Y. THOMPSON, S. K. KON, AND

E. H. MAWSON, Natl. Inst. for Res. in Dairying, Univ. of Reading, England. Abstracts of papers read at the Proc. of the Biochem. Soc., Nov. 7, 1942, London. Biochem. Jour., *36*, Nos. 10, 11 & 12 (combined): xvii. 1942.

The authors "have chromatographed numerous samples of human milk fat and have found that β-carotene contributes from 20 to 25% or even less of the total absorption at 451 mμ. In the majority of samples examined a pink band lies above that of β-carotene, chromatographically homogeneous with and showing the same absorption curve as crystalline lycopene, obtained from tomatoes.

"Shorthorn butter fat showed no lycopene zone. . . . In Guernsey fat there were three absorption zones between those of β-carotene and xanthophylls, one of which was pink and occupied the lycopene position. When 80 pounds of fresh tomatoes were given to a Guernsey cow during 6 days, the intensity of these zones rose from 4 to 16% of the total absorption. The pigment was identified as lycopene. In Guernsey milk β-carotene forms a smaller proportion of the total pigments than in Shorthorn milk."

A.O.C.

HERD MANAGEMENT

103. Dairy Management Problems. EARLE L. MOFFITT, Penn. State College. Milk Dealer, *32*, No. 11: 32, 66–67. Aug., 1943.

Stating "that 95% of the success in operating a farm comes from the thought or headwork put into the business," the author stresses the factors essential for financial success. The need of accurate farm records, the use of the data such records reveal, and the careful account of all details mark the difference between success and failure. Farm management studies made by the Pennsylvania State College are cited that show that unless yearly farm sales equal at least 25% of the total farm investment, there is practically no chance to show a yearly profit. Values of $15 to $18 per acre of crops on a general farm and $25 to $35 per acre of crops on special farms are given as goals to attain if a profit is to be assured. Details of dairy farm management are itemized and the proper balance to maintain in accord with proven practices are cited. The need of proper allocation of time and effort to each phase of farm management is pointed out. C.S.T.

ICE CREAM

104. Dried Whole Egg Powder. VI. Effect of Storage Temperature and Gas Packing on Keeping Quality. W. HAROLD WHITE, M. W. THISTLE, AND MARGARET REID, Natl. Res. Labs., Ottawa. Canad. Jour. Res., *D*, *21*, No. 9: 271. 1943.

Dried whole egg powders were obtained from three different manufacturers and stored at temperatures of 45°, 60°, 75° and 90° F. for periods up

to six months. Quality was assessed by determination of fluorescence and potassium chloride values. At 75° F. the rate of deterioration was comparatively rapid and at 90° F. it was markedly so. To maintain quality during storage dried egg should be stored at a temperature of 60° F. or lower.

The effect on keeping quality of packing in nitrogen, carbon dioxide, under vacuum, or in the form of compressed tablets was studied. Carbon dioxide alone had beneficial effect. O.R.I.

105. Dried Whole Egg Powder. VII. Effect of Temperature and Moisture on the Bacterial Content of Liquid and Dried Egg. N. E. GIBBONS AND C. O. FULTON, Natl. Res. Labs., Ottawa. Canad. Jour. Res., *D, 21,* No. 10: 332. 1943.

The bacterial content of liquid egg increased rapidly after about 6 hours at 68° F., 12 hours at 60° F., 25 hours at 52° F. and two or three days at 45° F. At 38° F. there was little change for five or six days, followed by a very gradual increase.

The bacterial content of the dried egg powder was influenced by the number of bacteria in the melange, the drying temperature, the rate of cooling, the storage temperature and the moisture content. Low drying temperature and rapid cooling of the powder favored survival. On storage the bacterial mortality increased with increasing time and temperature. Up to 8.6%, moisture content had little effect on bacterial survival. At moisture levels above 5% there was an increase in the number of molds, particularly at 75° and 90° F. O.R.I.

106. Acid Standardization. A. D. BURKE, Ala. Polytechnic Inst., Auburn, Ala. Ice Cream Field, *43,* No. 3: 64. 1943.

Early in the history of the ice cream industry it was considered good practice to "ripen" ice cream mixes and that a "livery" mix was believed to be the secret of good whipping, high yield and smooth-bodied ice cream. Experimenters early proved the fallacy of this concept and further showed that aging had little advantage beyond 24 hours.

The practice of adding certain salts to ice cream mixes as a basis of controlling mix viscosity, the author considers was the start of acid standardization. Five objections to "neutralization" are given but the author concludes that there are conditions which justify acid standardization or "neutralization."

Advantages listed for acid standardization are as follows: (1) provides uniform control of viscosity, (2) aids in whipping properties of high acid mixes, (3) improves melt down qualities of high acid mixes, (4) it helps intensify the apparent sweetness and (5) it may be of some benefit in retarding sandiness.

The author warns against over neutralization but considers it safe to reduce the acidity of ice cream mixes to about 0.18%. He suggests the following basis of calculating the correct value for acid standardization:

$$\frac{\text{Serum solids of mix}}{\text{Serum solids of milk}} \times \text{acidity of milk} = \begin{cases} \text{desired} \\ \text{standardized} \\ \text{acidity} \end{cases}$$

Directions are given for "neutralization" dependent upon weight of mix, its original acidity and the neutralizer used. W.C.C.

107. Bugaboo of Barriers. HAROLD F. PIERCE, London, Ontario. Ice Cream Field, *43*, No. 3: 42. 1943.

Many of the practices necessitated by the war have been beneficial to the ice cream industry of Canada. Costs have been reduced because of territory exchanges, reduced dealer service and cabinet repairs as well as marked reduction in advertising it is claimed. Hope is expressed that certain of the advantageous practices will continue after the war.

"Bootlegging" has been a minor problem. Strict adherence to quotas has been practiced. Cereal substitutes for dried milk have been used and sugar substitutes such as honey and corn syrup are officially tabooed. Glucose, although still allowed, is practically unavailable.

Regret is expressed that advertising has been so drastically reduced. The author concludes that except for the question of good-will building, dealer supervision, advertising and dealer help the Canadian picture is satisfactory. W.C.C.

108. What Other Solids to Use? The Serum Solids Situation. C. C. FLORA, Va. Polytechnic Inst., Blacksburg, Va. Ice Cream Field, *43*, No. 3: 19. 1943.

The author briefly discusses some of the problems resulting from wartime restrictions in the ice cream industry. He reviews the findings of various investigators and commercial operators as to what products can be used to replace serum solids in ice cream. He mentions a few of his findings which in general confirm those previously reported.

It is concluded that corn sugars, apple juices and syrups "offer possibilities for replacing part of the serum solids in ice cream." Oat flour (Avenex), Cincrose, soybean flour and wheat flour are also placed in this same category. W.C.C.

109. Serum Solids Substitutes. C. D. DAHLE AND D. V. JOSEPHSON, Pennsylvania State Col., State College, Pa. Ice Cream Field, *43*, No. 3: 14. 1943.

Experimental mixes which contained 10.5% fat, 8.4% serum solids, 15% sugar 60% of which was Frodex and 0.4% gelatin were prepared with vari-

ous flours by pasteurizing them at 160° F. for 20 minutes, since this was found to give better results than heating at 150° F. for 30 minutes. The results obtained confirmed the findings of others and further showed that Avenex or oat flour when used to the extent of 1% "gave as good or better results than 2% of the others."

They conclude that when over 1% oat flour is used a cereal flavor results but 2% soya flour also imparts a cereal flavor, whereas 2% of wheat, cake, or corn flour "caused no particular flavor defect in vanilla ice cream." Even "3 or 4% of ordinary wheat flour gave little indication of cereal taste."

The authors report that nothing except egg yolk and monoglyceride was found to improve overrun.

It is recommended that mixes containing cereal products be well agitated when used to insure the proper dispersion of part of the cereal which settles during standing. W.C.C.

MILK

110. Calculating Total Solids in Milk. CHARLES W. LIVAK. Natl. Butter and Cheese Jour., *35*, No. 1: 13. Jan., 1944.

Samples of milk were taken in November, December and January and analyzed for fat and solids by the Mojonnier method. Specific gravity measurements were made with lactometers and a Westphal balance. Percentages of total solids were calculated by Sharp and Hart's equation. The total solids so calculated were 0.228 and 0.307% higher than the Mojonnier method indicated, depending upon whether Rueda's or a "theoretical" correction factor was used to convert specific gravity values to 86° F. with reference to water at 86° F. W.V.P.

111. Why Certified Milk Should be Retained. J. HOWARD BROWN, Ph.D., Sc.D., Chairman, Council of the Amer. Assoc. of Med. Milk Commrs. Milk Dealer, *32*, No. 10: 48–49. July, 1943.

The possibility of the elimination of certified milk by allowing only two grades of milk as a war measure is discussed and a plea is made for retention of "certified milk" as a medicinal food rather than a premium milk. Certified milk is controlled by the American Association of Medical Milk Commissions, Inc., a non-profit organization. This milk is not produced and marketed for financial profit and its methods and standards of production are rigorously controlled. Nutritional, chemical and sanitary differences are maintained as are special qualifications of herds licensed to produce such milk. Ninety per cent of the sales of certified milk are made upon the prescription or recommendations of qualified physicians who consider it as a product "which fills a need and exerts an influence far beyond what its

volume of production and consumption might seem to justify." Its continuation as such a distinct product is urged. C.S.T.

112. Plant Cooling System for Milk Dealers. ANONYMOUS. Milk Dealer, *32*, No. 10: 32–33. July, 1943.

The adaptation of the cooling of water for refrigeration purposes to apply to flat-top dairy plants by use of a spray canopy system is recommended as a means of controlling and reducing plant building temperatures and as an auxiliary air-conditioning aid. The system advocated is by means of feed water pipes installed above the roof rather than under it, so that pipes require no insulation and can be operated with waste water from condenser coils at a nozzle pressure of 7 pounds. A spray pond covering the whole roof to protect it from solar heat, with a circulating system providing for recirculation, properly installed will reduce afternoon temperatures within the plant of a one-story flat-roofed building from 10 to 15 degrees. Such a system will also increase the life of the roof by stopping excessive expansion and contraction of roof material and loss of volatile oils from asphalt by hot sun. C.S.T.

113. Making Quality E asy for Milk Producers. PAUL H. MONDT. Milk Dealer, *32*, No. 11: 30–31. Aug., 1943.

Farm labor as well as plant labor is critical. Therefore the producer needs all the help the plant operator can extend to promote continued production of quality milk. The producer should be educated as to the need of quality to prevent waste, promote edibility and use of dairy products, and to provide extra food. This can be done by means of posters, loaning such items as hair clippers, and by distributing whitewashing formulas and directions to farmers. Directions for the proper care of milking machines; blueprints on correct milk house construction, together with cement or building instructions; providing building forms for cooling tanks; aid in the purchase of supplies, cans, brushes, powders, etc.; and suggestions for the correct care of the cows, equipment and milk are discussed. C.S.T.

114. Consumer's Problems of Every-Other-Day Milk Delivery. GEORGE F. DOW. Milk Dealer, *32*, No. 12: 33–34, 66–68. Sept., 1943.

The "other side," namely, the consumer's rather than the distributor's problems of every-other-day milk delivery is presented. Such factors as the keeping quality of milk, home refrigeration facilities and changes in source of supply are analyzed from a survey of 966 families in Portland and Westbrook, Maine. Consumer problems were souring of milk in hot weather, off-flavor development from standing outside or uncapped in home refrigerators, thickened cream layer, poorer keeping quality of raw milk as com-

pared with pasteurized, and the relation of time of delivery to the keeping quality of the milk delivered. The type of home storage of the milk was a factor in that ice refrigeration as compared with mechanical presented more of a quality and keeping problem. Detailed percentages of various complaints are given. On the whole the net change in milk purchased and consumed was negligible; quality was unaffected if adequate refrigeration was available in homes and if the time of delivery was adjusted. Seventy-six per cent received their milk from distributors with established home deliveries and but few changed dealers, thus demonstrating that consumers were willing to cooperate with the plan to conserve trucks and labor as a war measure. C.S.T.

115. **Pioneer Milk Pasteurizing Plant.** FRED W. MEEN. Milk Dealer, *33*, No. 2: 39–40. Nov., 1943.

The description of equipment, methods used and of delivery and sales methods followed in the organization and starting of what is said to be the first commercial bottled fluid milk pasteurization plant established in the United States at Rush City, Minnesota, in 1897, is of historical interest to the milk industry. C.S.T.

116. **Securing Customer Cooperation to Ease Milk Delivery Problem.** C. W. ESMOND, G. P. Gundlach & Co., Cincinnati, Ohio. Milk Dealer, *33*, No. 2: 48–50. Nov., 1943.

With the necessarily curtailed service to all milk customers in effect as a wartime emergency measure, the need for customer cooperation—always essential for best dealer-customer relationship—is now more than ever a "must" if confusion, complaints and loss of customers are to be avoided. An intelligent, systematic and continuous educational program to acquaint the customer with the problems confronting the dealer together with a recognition of the consumer's unaccustomed inconveniences and problems will go far towards solving present-day wartime milk delivery problems, as well as to meet post-war competition and "to retain and to improve upon all the economical distribution methods of war time, such as the elimination of special delivery, standing orders, conservation of bottles, reduced number of deliveries per week, minimizing the overlapping of routes, collecting at lowest possible cost, saving unnecessary steps for milkmen, etc.," and thus retain and maintain the closer and more friendly dealer-customer cooperation developed as a wartime necessity. C.S.T.

117. **Improving Chocolate Drinks.** B. E. HORRALL, Purdue Agr. Expt. Sta., Lafayette, Ind., AND M. O. MAUGHAN, Amer. Dry Milk Inst., Chicago, Ill. Milk Dealer, *33*, No. 3: 24–25, 48. Dec., 1943.

A survey cited by the authors revealed that 80% of all chocolate milk sales was a "plus" not a "replacement" volume, indicating that chocolate

increases the palatability of milk to many consumers. Studies therefore were undertaken "to (a) improve the flavor and (b) increase the nutritive value of chocolate drinks." Experimental technique was to use various percentages of milk fat where milk solids-not-fat were added to different lots of whole and separated milks plus four different chocolate products and sugar. Cream, butter and butteroil were used for desired fat combinations and spray-dried skim milk powder for solids-not-fat additions. Six experiments were conducted with varying percentages of milk fat to which added milk solids-not-fat were combined. All samples were tested by seven or more judges. Added milk solids-not-fat to the extent of 3% improved all commercial chocolate milk drinks. A 2% butterfat product enriched with 3% milk solids-not-fat was adjudged the best-flavored product, followed closely by the 3% and 1% butterfat products to which 3% milk solids-not-fat were added. A commercial formula for addition of spray-dried skim milk powder to either a skim milk or reconstituted milk mixture is given. C.S.T.

118. The Function of the Laboratory in the Control of Milk Supplies. F. W. FABIAN, Res. Prof. Bact., Mich. State Col., East Lansing, Mich. Jour. Milk Technol., *6*, No. 5: 278. Sept.–Oct., 1943.

A good discussion is given of the various laboratory tests used in the dairy industry and their relative values in determining the sanitary quality of milk.

The author concludes that "it looks as though we are going to use the plate method less since it is expensive, time-consuming and tells only a part of the story. In its stead we shall substitute the direct microscopic test supported by other tests. For raw milk, we shall use the odor test, the sediment test, and either the direct microscopic or the resazurin test or both. For pasteurized milk, we shall use the direct microscopic method supplemented by the coliform and phosphatase tests."

The author believes it will be some time yet before laboratory tests will be developed to the stage where they will supplant the milk inspector. He does think, however, that the future inspector will be better trained and will be a combination inspector and laboratory man, doing most of his own laboratory work.

The ideal situation is to use the milk inspector to locate the visible dirt and the laboratory to find the invisible dirt. "They should supplement rather than supplant each other." L.H.B.

119. Care of Milking Machines. C. K. JOHNS, Central Expt. Farm, Ottawa, Canada. Jour. Milk Technol., *6*, No. 5: 274. Sept.–Oct., 1943.

In studies made at the Central Experiment Farm, it was found possible to keep milking machine rubber parts in good sanitary condition without

the use of hot water after each milking. This method was developed in 1930 and has been in use ever since.

During a period of nearly twelve years, more than 1200 samples of the mixed raw milk (nights and mornings) were taken from the pasteurizing vat prior to pasteurization. More than 70% of the agar plate counts were below 10,000 per ml. and more than 95% were below 50,000 per ml.

The method used is very simple and is as follows:

Immediately after milking at least two gallons of clean, cold or lukewarm water are drawn through each unit, raising and lowering the teat cups several times to obtain an air-brush effect. The outer surfaces of the teat cup assembly are then cleaned off with a brush and hot detergent, hung on a solution rack and filled with a weak (0.5%) lye solution until the next milking. At that time the solution is drained out and the units re-assembled and ready for use. It has not been found necessary to rinse the units to remove traces of lye solution, but a chlorine rinse at this time could be drawn through the unit to advantage. At intervals of one or two weeks, the rubber parts are dismantled, primarily to prevent adherence to the metal, and for thorough inspection of the tubing and liners to note their condition. Worn out tubing and liners are replaced at this time, and if necessary, liners are trimmed to proper length. The tubes and liners are brushed in hot detergent solution and rinsed before re-assembling.

The lye solution has an advantage over the chlorine soak solution in that the lye saponifies any butterfat and dissolves any casein which might be present, thus leaving the rubber parts physically clean. The lye solution is also a good germicide and prevents the growth of bacteria in the tubes.

L.H.B.

MISCELLANEOUS

120. Proper Paint Helps Solve Surface Maintenance Problems. ANONYMOUS. Milk Dealer, *32*, No. 11: 29–58. Aug., 1943.

Stressing the need of proper maintenance of all milk plant equipment, the paint-up program, with paint still available, can and should be regularly maintained, particularly since the milk plant must of necessity operate under conditions of moisture and steam condensation especially destructive to ordinary paint.

The need of special moisture and acid-resistant protection from corrosion and fungus and mold growths by use of paints possessing such properties is stressed. The use of proper white and light-tinted paints for increased illumination which reduces eye strain and fatigue, aids employee morale and decreases accidents. In some plants accidents have been eliminated 100% by selection of a high light-reflecting paint. A table of reflection values for different colors ranging from 11 to 25% for dark green to 80–89%

for white is given. The suggestion is made that wherever practical, increased light reflection is obtained by painting floors white or light colored. C.S.T.

121. First Aid for Ailing Cold Storage Rooms. ANONYMOUS. Milk Dealer, *32*, No. 11: 26. Aug., 1943.

Citing a recent survey made by the Refrigeration Service Engineers Society that approximately half of the refrigeration service men have left their former jobs, the author stresses the need for all fluid milk plants to keep their refrigerating machinery and cold storage rooms at top efficiency lest spoiled products through breakdowns with resulting higher operating costs result. A new type of circulating fan which requires no skilled mechanic to install provides for better air circulation on a new principle of blowing the cold air from the refrigerated coils up to the ceiling, down the walls and up the center, moving all the air in the room to maintain an even and equalized temperature. Such a movement of air promotes dryness, dispels odors quickly, prevents frosting of coils, retards mold growth and reduces cost. A diagram of air currents after installation of fan graphically illustrates diffusion of air. C.S.T.

122. National Control or Free Competition for Britain's Milk Industry. GEORGE WALWORTH. Milk Dealer, *33*, No. 2: 64–66. Nov., 1943.

Whether government control of Britain's milk industry after the war is made permanent or whether the industry is permitted to revert to pre-war free competition is contingent upon the extent to which the present essential processing and price-control measures are voluntarily retained by private enterprise. To insure national nutritional requirements, proper price relationship as to milk quality and its utilization, the maintenance of proper balance between liquid milk and processed products consumption, economical maintenance of sales and distribution and milk quality control are factors the British milk industry must face and solve satisfactorily to avoid national post-war control of milk. The establishment of a milk control council by the industry with power to regulate such factors in the interest of consumers, as well as to insure adequate returns to the industry, is advocated. This British question is of importance to the United States in that post-war international relationships are involved and concern all milk-producing nations. "Liquid milk occupies the key position in Britain's agriculture but dairy produce will be of considerable significance in overseas trading with Britain." C.S.T.

123. Use of Chlorine on the Farm. JACK KEENAN, Gen. Lab. Div., Penna. Salt Mfg. Co., Philadelphia, Pa. Milk Dealer, *33*, No. 2: 52–57. Nov., 1943.

Since milk quality control starts at the farm, the utilization of chlorine

for cleaning all utensils, for washing cows and milkers' hands, rinsing cans, etc., is of vital importance. Methods of preparing standard chlorine solutions and rinses are given for all such uses. Stress is laid upon need of using chlorine in washing cows' udders and teats to prevent infection, and in cleaning milking machines to prevent spread of disease. The author concludes that "next to sunshine, chlorine may be said to be our best bactericide" and that its use is safe, inexpensive and particularly adapted to use on the farm. C.S.T.

124. Plastics. ANONYMOUS. Milk Dealer, *33*, No. 3: 26–27. Dec., 1943.

With the advent of war, substitute materials for use in dairy plant equipment became necessary. Since then substitutes for substitutes have appeared. The use of plastics, since they are light, unbreakable, clear and impart no flavor or odor, in making both old and new milk plant equipment, would seem to afford highly interesting possibilities. Particularly interesting is the possibility of supplanting standard tubing and piping with plastic material. C.S.T.

125. Tin in the Dairy Industry. JULIA DEXTER, Battelle Memorial Inst., Columbus, Ohio. Milk Dealer, *33*, No. 3: 34, 46–48. Dec., 1943.

The use of tin in the dairy industry from the tin milk pail of the farmer to the tin can on the grocery store shelf through all the intermediate steps of collection, transportation, cooling, storing and packaging in tin-coated equipment is described and its present critical status discussed. New methods of utilizing tin as a coating which will favor retention of tin in postwar use in the dairy industry are described. Electrolytic plating of tin by different new methods; the use of white bronze plating to tin copper or in combination with nickel, tin spraying and new and better tinfoil for wrapping dairy products are postwar possibilities which "point the way to better and far heavier tin coatings than have been possible in the past. Repairs and relining are facilitated in many cases by electrolytic tinning means. Tin-coated steel is the most inexpensive material of construction for dairy equipment." C.S.T.

126. Steel for Insulation. JAMES G. MACORMACK, Amer. Flange & Mfg. Co., Inc., and C. T. Hogan & Co., Inc., New York. Ice Cream Field, *43*, No. 3: 34. 1943.

The author claims that Ferro-Therm steel insulation offers increased efficiency and economy to the ice cream industry. Ferro-Therm steel insulation sheets are made of thin gauge commercial steel with a hot dipped lead and tin alloy coating "which reflects 90 to 95% of radiant heat. These sheets are installed with approximately one-half inch air space on either

side, broken up by separators every 24 to 32 inches. Ferro-Therm steel insulation was first introduced in 1933–34 but it has been extensively used since 1937 in many industries requiring refrigeration.

Eight sheets of Ferro-Therm insulation in an overall thickness of 4 inches is equivalent to 8 inches of corkboard. Ferro-Therm sheets from 26 U.S.S. gauge to 18 U.S.S. gauge are used for the outside linings for rigidity, while all intermediate steel sheets are No. 38 U.S.S. gauge and weigh 0.25 pound per square foot.

Fifteen sheets within a thickness of 6 inches used on a "dry ice" storage cabinet resulted in a loss of 1.8% for 24 hours whereas 8 inches of corkboard insulation on the same type of cabinet resulted in 3% loss per 24 hours. The Ferro-Therm container weighed 1100 pounds and held 2938 pounds of dry ice, whereas the corkboard container weighed 1500 pounds and held 2250 pounds of dry ice.

The degree of luster or optical brightness has little to do with the ability of the surface to reflect heat or infra red rays. Tests by the U. S. Bureau of Standards show that reflective insulating sheets exposed to industrial atmosphere for two years until they became a dull gray-black had 97% of the reflectivity of radiant heat they had when new and bright. Moisture condensation is very slight in a Ferro-Therm construction. The heat storage capacity of the steel insulation is relatively low, which permits more rapid temperature pull down than with the usual type of mass insulation.

W.C.C.

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Processing

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Food processing falls into three groups. Sometimes it means a process of subtracting. Sometimes a process of adding essential ingredients. And sometimes applying a "treatment" that neither adds nor subtracts.

Of course, sugar refining is a good example of the subtracting process, because many things have to be removed by chemical means from the cane or beets to get the pure, white sugar crystals. . . . Vanilla flavoring is an excellent example of the adding process, because vanillin has to be added to the artificially cured vanilla beans to give the full-rounded vanilla flavor to the finished product. . . . And certainly pasteurized milk is the perfect example of processing that calls for neither adding nor subtracting.

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Heat and cold also play important roles in bringing vanilla ice cream to the consumer. Heat is part of the process used in curing the vanilla bean. Cold is very much in evidence because the ingredients must be subjected to man-made low temperatures to produce ice cream. And to offset the effect of the artificial freezing—vanillin must be added to the cured vanilla beans to give the finest flavor.

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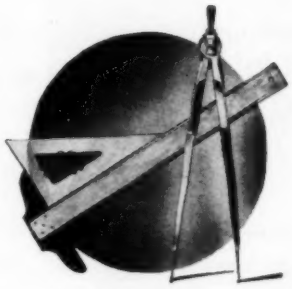

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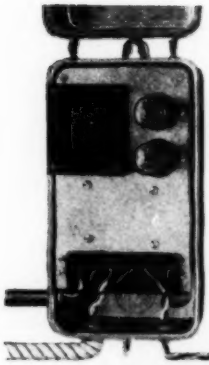

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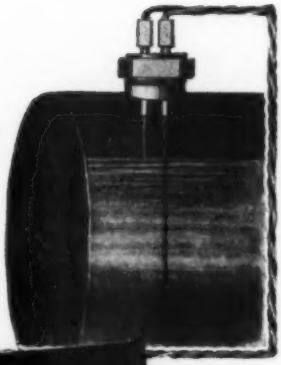

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